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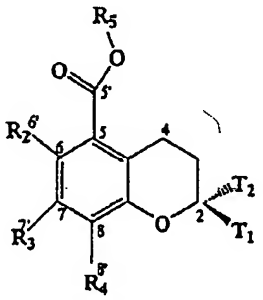
(54) Title: CARBOXYBENZOPYRAN DERIVATIVES AND COMPOSITIONS

(57) Abstract: Benzopyran derivatives including carboxylbenzopyran derivatives are described. Compositions that include the benzopyran derivatives are also provided.



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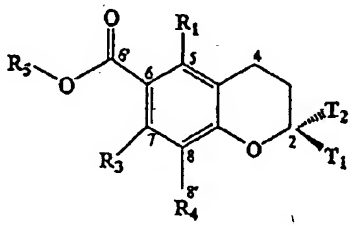

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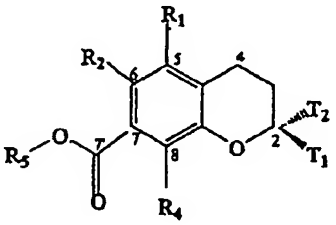

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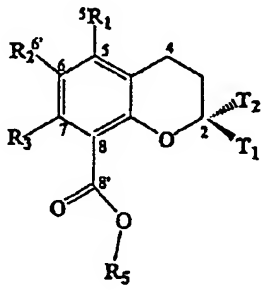

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WO 02/076938 A2

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CARBOXYBENZOPYRAN DERIVATIVES AND COMPOSITIONS

FIELD OF THE INVENTION

The present invention relates to carboxybenzopyran derivatives and compositions including the derivatives.

BACKGROUND OF THE INVENTION

Benzopyrans are an important class of molecules that includes vitamin E, tocopherols and tocotrienols. These compounds have been developed to improve the water solubility of the oily vitamin so as to improve dietary or parenteral uptake of vitamin E in certain clinical and veterinary conditions, to discover novel medicaments, and to develop novel antioxidants. Only recently, the solubilization properties of tocopherols, tocopherol acetate, tocopherol succinate, TPGS and tocotrienols have been recognized. However, the chemistry of tocopherol derivatization for this purpose has not advanced much since the introduction of TPGS in 1951, and has not encompassed use of carboxylated, carbonated, or carbamated benzopyrans as efficient starting materials for derivatization. Previous work has not recognized the use of these derivatives as surfactants, or as pharmaceutical excipients in emulsions, nanoemulsions, microemulsions, liposomes or micellar solutions.

The present invention seeks to fulfill this need and provides further related advantages.

SUMMARY OF THE INVENTION

In one aspect the present invention provides benzopyran derivatives. In one embodiment, the benzopyran derivatives are carboxylate derivatives. In another embodiment, the benzopyran derivatives are amide derivatives. In a further embodiment, the benzopyran derivatives are acetic acid derivatives. In another embodiment, the benzopyran derivatives are acetamide derivatives. In yet another embodiment, the benzopyran derivatives are carbonate derivatives. In a further embodiment, the benzopyran derivatives are carbamate derivatives.

In another aspect of the invention, compositions that include the benzopyran derivatives are provided.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to

the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

FIGURES 1A-D are chemical structures of representative benzopyran carboxylate derivatives of the invention;

FIGURES 2A-D are chemical structures of representative benzopyran amide derivatives of the invention;

FIGURES 3A-D are chemical structures of representative benzopyran acetic acid derivatives of the invention;

FIGURES 4A-D are chemical structures of representative benzopyran acetamide derivatives of the invention;

FIGURE 5 is the chemical structure of vitamin E;

FIGURE 6 is the chemical structure of d-α-tocotrienol;

FIGURE 7 is the chemical structure of 5-carboxy-d-α-tocopherol;

FIGURE 8 is the chemical structure of 6'-O-d-δ-tocopherol acetic acid ester;

FIGURE 9 is the chemical structure of a representative tocoglycinate of the present invention;

FIGURE 10 is the chemical structure of a representative tocoglutamate of the present invention;

FIGURE 11 is the chemical structure of a representative tocoglutamine of the present invention;

FIGURE 12 is a schematic illustration of the synthesis of 5-bromo-d-δ-tocopheryl-6-ol;

FIGURE 13 is a schematic illustration of the synthesis of 5-carboxy-d-δ-tocopheryl-6-ol; and

FIGURE 14 is a schematic illustration of the synthesis of RRR-d-α-tocopheryl-6-carboxylic acid.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

In one aspect the present invention provides benzopyran derivatives. In one embodiment, the benzopyran derivatives are carboxylate derivatives. In another embodiment, the benzopyran derivatives are amide derivatives. In a further embodiment, the benzopyran derivatives are acetic acid derivatives. In another embodiment, the benzopyran derivatives are acetamide derivatives. In yet another embodiment, the

benzopyran derivatives are carbonate derivatives. In a further embodiment, the benzopyran derivatives are carbamate derivatives.

The chemical structures of representative tocopherol succinic acid derivatives are illustrated in FIGURES 1-4, 9-11, 13, and 14. FIGURES 1A-D are chemical structures of representative benzopyran carboxylate derivatives of the invention. FIGURES 2A-D are chemical structures of representative benzopyran amide derivatives of the invention. FIGURES 3A-D are chemical structures of representative benzopyran acetic acid derivatives of the invention. FIGURES 4A-D are chemical structures of representative benzopyran acetamide derivatives of the invention. FIGURE 9 is the chemical structure of a representative tocoglycinate of the present invention. FIGURE 10 is the chemical structure of a representative tocoglutamate of the present invention. FIGURE 11 is the chemical structure of a representative tocoglutamine of the present invention.

In these figures, the noted substituents are as described below.

R_1, R_2, R_3, and R_4 are substituents selected independently from hydrogen, hydrocarbyl, amino, hydroxyl, and carboxyl. In one embodiment, these substiuents are selected from hydrogen, C_1-C_4 hydrocarbyl, carboxyl, and hydroxyl. In another embodiment, the substiuents are selected from hydrogen, methyl, and ethyl.

R_5 is a substituent that associates with water to form at least 2 hydrogen bonds, in one embodiment 3 hydrogen bonds, and in other embodiments from 2 to about 200 hydrogen bonds. R_5 can optionally form a salt in buffered water or saline, and may be selected from a carboxylate (such as sorbate, tartarate, succinate, citrate, gluconate, glucoheptonate, glycerate, itaconate, aconitate, galacturonate, galactarate, glutarate, creatine, fumarate and its polymers, ascorbate, lactate and its polymers, pangamate, pantothenate, or para-aminobenzoate), an amine (such as glucamine, glucosamine, thiamine or choline), a phosphate (such as glycerophosphate, fructose-6-phosphate or phosphocreatine), an amino acid, a purine (such as adenine and guanine), a nucleoside or nucleotide (such as adenosine, deoxyadenosine, guanidine, cytosine, thymidine, uridine, or polyadenosine), a polypeptide (such as vasopressin, polypep, ferrichromes, oxytocin, or rifampin), an alcohol (such as erythritol, adonitol, ribiflavine, flavine-adenine-dinucleotide, glucovanillin, taurocholate, glycocholate, tauroursocholic acid, glyco-nor-ursodeoxycholic acid, thiol, glycerol, mannitol or cyanocobalamin), a sugar (such as glucosamine, n-acetylglucosamine, n-acetylneuraminate, lactose, ribose, arabinose, rhamnose, raffinose, maltose, lactobionose, heparin sulfate, trehalose, gluconate,

galactose, sucrose or glucose), a polyhydric alcohol (for example $-(OCH_2OH)_nOH$, $-(OCH_2CHOH)_nOH$, $-(OCH_2CHOHCHOH)_nOH$, $-(OCH_2CH_2CHOH)_nOH$, $-(OCH_2CHOH)_nOH$, $-(OCH_2CH_2CHOH)_nOH$, or $-(OCH_2CHOHCH_2)_nOH$, where n is 1 to 100, and branched or block co-polymers of the same). In certain embodiments, R_5 includes residues of carnitine, sarcosine, taurine, methionine, glutathione, β-alanine, glycine, glutamate, glutamine, aspartate, asparagine, ornithine, arginine, γ-aminobutyrate (GABA), serotonin, adrenaline, histamine, melatonin, tryptamine, alanylglutamine, glycylglutamine, glycylsarcosine, valyl-lysine, aspartylalanine, glutamyltryptophane, lysyl-sarcosine, glycylproline, triglycine, polyglutamate $(Glu)_n$, polyglutamine $(Gln)_n$, polyglycine $(Gly)_n$, polyalanine $(Ala)_n$, polyproline $(Pro)_n$, poly-$(GlyProAla)_n$, polyserine $(Ser)_n$, and other biogenic amines as defined below, polyesters, copolymers of succinate, glycerol, and polyethylene glycol, polyhydroxyalkonates, polyhydroxyproprionate, poly-(3-hydroxyvalerate), poly-(3-hydroxyhexanoate), poly-(4-hydroxyvalerate), poly-(5-hydroxyvalerate), generally R-3-hydroxyacid polymers and their derivatives, polyglycolides (PGA), polylactides (PLA), substituted polyhydroxybutyrates, folate, glycogen, chitosan, dextran, dextrin, gluconate, poly-N-substituted glycines, polyvinylpyrrolidinone, poloxamer, polyvinylalcohol, polyethylene glycol, l-amino-polyethyleneglycol, or composites (co-polymers) of the above.

In an alternate embodiment, R_5 provides a derivative that is not bonded or is weakly bonded by a C8 column packing, and most preferentially is not bonded or is weakly bonded by a C18 column packing (for example BondElute™). Bonding can be assessed by measuring retention times on an HPLC set up with a reverse phase column and a gradient solvent system progressing from relatively nonpolar to more polar. Those compounds that are poorly retained (i.e., have low retention times) are the preferred compounds for R_5. Molecular weights for R_5 are typically between 20 Da to 5 kDa. In one embodiment, R_5 molecular weights are from about 80 Da to 5000 Da. In another embodiment, R_5 molecular weights are from about 180 Da to 2500 Da.

T_1 is a C_1 to C_{80} hydrocarbyl, hydroxyhydrocarbyl, carboxyhydrocarbyl, oxyhydrocarbyl ketohydrocarbyl, oxohydrocarbyl, phosphohydroxy hydrocarbyl, saturated or unsaturated, branched or unbranched. In one embodiment, T_1 is an isoprenoid, terpene, diglyceride, or phospholipid. In another embodiment, T_1 is a phytyl (4,8,12-trimethyl-tridecyl) or trienyl (4,8,12-trimethyl-3,7,11-tridecatrienyl).

T_2 is hydrogen, or C_1 to C_{80} hydrocarbyl, optionally substituted, saturated or unsaturated, branched or unbranched, alkyl or aromatic. In one embodiment, T_2 is a C_1 to C_{18} hydrocarbyl group. In another embodiment, T_2 is methyl or ethyl. Alternatively, T_2 is a carboxyl group, wherein the carboxyl group is optionally esterified or amidated, respectively, with a C_1 to C_{80} alcohol or amine, preferably a C_1 to C_{18} alcohol or amine, and the alcohol or amine is optionally substituted, saturated or unsaturated, branched or unbranched, cyclical or acyclic.

The stereochemistry of T_1 and T_2 may be as d- or l-stereoisomers or as racemates, and the invention is not limited by the stereochemistry of any chiral centers.

The benzopyran derivatives can be cationic, anionic, zwitterionic, multipolar or nonionic. Ionically charged derivatives can be formed and used as salts, for example as the sodium, hydrochloride, citrate, lactobionate, propionate, succinate, potassium, lithium or palmitate salt.

The invention further relates to compositions in which a pharmaceutical, nutriceutical, cosmeceutical, vitamin, foodstuff, antigen, catalyst, cell, nanoparticle, oligonucleotide, gene, extract, cosmetic or fiber is solubilized, protected or dispersed in a solution, particle, emulsion, microemulsion, nanoemulsion, liposome, niosome, molecular matrix or coating comprising one or more of the benzopyran derivatives, optionally with other oils, co-solvents, surfactants and cosurfactants. In one embodiment, the composition is a biocompatible or therapeutic formulation comprising one or more benzopyran derivatives for application to a human or to an animal by any of a variety of routes, including but not limited to oral, topical and parenteral administration. Such compositions include solutions, suspensions, emulsions, emulsion preconcentrates, liquigels, lotions, astringents, soaps, ointments, toothpastes, topicals, capsules, sustained release granules, powders, tablets, nosedrops, eyedrops, excipients, sunscreens, surgical dressings, intravenous infusions, depot or sustained release injections, and coatings for prosthetic devices.

The benzopyran derivatives of the invention are 1-benzopyran derivatives and can be synthesized by chemistry that is commercially attractive. The benzopyran derivatives have unexpected utility as surfactants, as biocompatible surfactants, as pharmaceutical excipients, and as bioavailability enhancers. In contrast, typical commercial surfactants such as sodium oleate (a principal component soap), betaine, or SDS (sodium dodecyl sulfate) dissolve biological membranes and are corrosive to cells, so the utility of

surfactants that, paradoxically and unexpectedly, serve both as detergents and as stabilizers of biocellular membranes is anticipated to be great.

The benzopyran derivatives displayed surfactant properties of foaming and emulsion formation even before the protective groups on the "hydrophilic head" had been removed.

The biocompatibility of tocol oils in contact with cell membranes and organelles is truly remarkable, and it is well known that tocols characteristically stabilize biological membranes in the presence of other surfactants. The biocompatible benzopyran derivatives are surfactants that may be anionic, cationic, zwitterionic, multipolar, or non-ionic.

To assist in understanding the invention, the following definitions are provided.

Vitamin E: Vitamin E as used herein is the common name for RRR-α-tocopherol (d-α-tocopherol, *sensu stricto* 2,5,7,8-tetramethyl-2-(4',8',12'-trimethyltridecyl-6-benzopyranol), the vitamin named by Dr. George Calhoun and Dr. H.M. Evans in 1936. The suffix "-ol" denotes the presence of the 6-hydroxyl on the benzopyran ring. Vitamin E is a member of the family "tocopherols." Vitamin E has a bioequivalence of 1.00 αTE units in biological assays for Vitamin E activity.

Tocopherol(s): Tocopherols are a family of natural and synthetic compounds containing three key structural elements, a benzopyran ring, phenolic alcohol, and phytyl tail. Vitamin E is an important representative of the tocopherol family, and its molecular structure is shown in FIGURE 5. Not all tocopherols have three methyl groups on the chroman head. The simplest family member contains no methyl groups on the chroman ring, 6-hydroxy-2-methyl-2-phytylchroman), and is sometimes simply referred to as "tocol", although the term "tocol" is used herein to represent all tocopherols and tocotrienols. There are four tocopherol family members that are commonly encountered in food and natural products, and eight possible isomers in total (not including stereoisomers). The names of the family members are shown in the table below.

Position of methyl groups on chroman head	Tocopherol family common name
5,7,8	α-tocopherol
5,8	β-tocopherol
7,8	γ-tocopherol
5,7	ξ_2-tocopherol
8	δ-tocopherol
5	5-methyltocol, "ε_1-tocopherol"
7	η-tocopherol
0	"tocol"

It is important to recognize that all tocopherols share the phenolic alcohol as a functional group at the 6-position on the chroman head, regardless of the position of the methyl groups. In addition, the R/S stereoisomers described for the phytyl tail of α-tocopherol (3 chiral centers, 8 isomers in all) are also present in each of the other tocopherol families, e.g., beta, delta and gamma. Thus the total number of natural molecules named in the table, including stereoisomers, is 8 x 8 = 64.

Tocotrienol(s): Tocotrienols have structures related to the tocopherols, but possess a 3', 7', 11'-triene "tail" at the 2-position on the benzopyran ring. For illustration, the structure of d-α-tocotrienol is shown in FIGURE 6. Again, as is the case for the tocopherols, not all tocotrienol family members have three methyl groups on the chroman head. There are four family members that are commonly encountered in food, and eight possible members in total, more if the desmethyl forms are considered.

Tocotrienol nomenclature is not fully consistent at the level of common names. However, all tocotrienols share the phenolic alcohol at the 6-position on the chroman

head. Adding another layer of complexity, the double bonds at the 3, 7, and 11 positions of the tail may be "cis" or "trans", but typically are all-trans in the natural products.

Interestingly, the δ-tocopherol and δ-tocotrienols are some of the best antioxidants of the group.

Tocol-soluble: Refers to the property of certain molecules characterized as being soluble directly, or with the aid of a co-solvent, in a tocol. As an operative definition, the most useful way to determine tocol solubility is to dissolve the compound of interest in a tocol or to use a co-solvent such as ethanol.

U.S. Patent Application No. 09/671,753 filed September 27, 2000, and PCT application PCT/US00/26467, (both of which are hereby incorporated herein by reference) disclose formation of tocol-soluble ion pairs between charged tocol derivatives and oppositely charged therapeutic compounds. By "ion pair" is meant a neutral pair formed between two oppositely charged compounds. Compounds of the present invention are among the tocol derivatives that are capable of forming tocol-soluble ion pairs with such oppositely charged therapeutics, thereby rendering them soluble in tocols or enhancing existing tocol solubility. The resulting compositions can be incorporated into various types of pharmaceutical compositions, including multiphasic compositions or their precursors, such as emulsions, liquid crystalline gels, self-emulsifying drug delivery systems, or liposomal or niosomal dispersions, for oral or other (including parenteral) administration.

Tocan or Tocans: "Tocan" or "tocans" are used herein in a broad sense to indicate the various members of the families of tocopherols and tocotrienols, their rarer natural and synthetic analogs, and in addition all benzopyran derivatives substituted at the 2-position by T_1 and T_2, where T_1 is a C_1 to C_{80} hydrocarbyl, hydroxyhydrocarbyl, oxyhydrocarbyl, carboxyhydrocarbyl or phosphohydroxy hydrocarbyl, saturated or unsaturated, branched or unbranched, an isoprenoid, terpene, diglyceride, phospholipid, a phytyl (4,8,12-trimethyl-tridecyl), or trienyl (4,8,12-trimethyl-3,7,11-tridecatrienyl), and T_2 is a hydrogen, halo, hydrocarbyl, carbonyl, methyl, ethyl, or carboxyl, as the l-stereoisomer.

Tocans also include tocols esterified at the 6-hydroxyl on the benzopyran ring. When administered *in vivo*, these derivatives are readily de-esterified at low pH or by esterases in the thoracic duct and in the blood, releasing the free tocol. Common tocol esters known in the art include the acetate, succinate, maleate, phosphate, linoleate,

nicotinate, ascorbate, retinoate, quinone, and a pegylated diester derivative known as TPGS (tocopherol polyethylene glycol succinate).

A special case is the benzopyran derivative known as 6-hydroxy, 2,5,7,8-tetramethylchroman-2-carboxylic acid (commercially available as Trolox®), which has a reactive carboxyl functional group at the two position and is relatively hydrophobic despite lack of a lipid sidechain at T_2. Tocol desmethyl analogs have been isolated or synthesized. Other synthetic tocols include Raloxifast.

5-d-α-tocopherol carboxylate: has the chemical structure shown in FIGURE 7.

6'-O-d-δ-tocopherol acetic acid ether: has the chemical structure shown in FIGURE 8.

Tocoglycinates: representative benzopyran derivatives of the invention having the chemical structure shown in FIGURE 9, where R_1, R_2, R_3, R_5, T_1, and T_2 are as previously defined.

Tocoglutamates: representative benzopyran derivatives of the invention having the chemical structure shown in FIGURE 10, where R_1, R_2, R_3, R_5, T_1, and T_2 are as previously defined. Both alpha- and gamma-carboxyl tocan derivatives, and dendrimeric structures (Glu_n) and polymers (Glu_n) of glutamate (where n = 2 to 50) are provided.

Tocoglutamines: representative benzopyran derivatives of the invention having the chemical structure shown in FIGURE 11, where R_1, R_2, R_3, R_5, T_1, and T_2 are as previously defined. Dendrimeric structures (Gln_n) and polymers (Gln_n) of glutamate (where n = 2 to 50) are provided.

Linker: In chemical synthesis, conjugations of two molecules A and B may take place using a linker "C" to modify the reactivity of a functional group so that the joining takes the form A+C+B in the final structure. Note the position of C between A and B. Linkers may be homo- or heterobifunctional, or multifunctional as in the synthesis of dendrimeric molecules.

Spacer: A spacer is a special class of linkers in which the separation of A and B is increased by the length of the spacer C.

Cap: An end group on a side chain, particularly on a polymer.

Surfactant: Surfactants are bipolar molecules characterized by one simple property: they are driven to occupy interfaces between two phases, typically either liquid/gas phase interfaces, liquid/solid interfaces, or liquid/liquid phase interfaces (for immiscible liquids), so that the free energy of the boundary surface between phases is

reduced. Obviously, the "surfactanticity" of any molecule is not independent of the properties of the immiscible phases under study. With respect, for example, to water and oil, a surfactant will contain both a water-loving "head" and an oil-loving "tail". Other surfactants, however, have fluorophilic "tails." The basic principle is that the molecule is amphipolar with respect to the two phases with which it associates. Surfactants may be classed into five sub-groups: anionic, cationic, zwitterionic, multipolar and non-ionic on the basis of chemical structure and the presence or absence of electrostatically charged substituents. Surfactants are also sometimes termed "emulsifiers." Catanionic, gemini, and bolaform surfactants are special cases.

Co-surfactant: A second surfactant that aids in reduction of the surface free energy between two phases, most typically that aids in fine emulsification of an oil and water or dirt and water system.

Hydrophile-Lipophile Balance (HLB): Is an empirical formula used to index the relative detergency of surfactants. Its value varies from 1 to about 45 and in the case of non-ionic surfactants from about 1-20. In general, for lipophilic surfactants the HLB is less than 10 and for hydrophilic ones the HLB is greater than 10.

Critical Micellar Concentration (CMC): Is an experimentally determined concentration of amphiphile, surfactant or detergent molecules in solution distinguished by the appearance of organized "micelles" (defined below).

Oil: Any of a class of hydrocarbon derivatives that are hydrophobic and immiscible or poorly miscible with water. They may be synthetic or derived from plants, animals or microorganisms. Such oils include "grease," waxes, "dirt," triglycerides, diglycerides, derivatives of mono- and diglycerides, essential oils, Vitamin oils, nutrient oils, squalene, squalane, waxes, terpenes, ethers and crown ethers, and may be either synthetic or natural. In general, the melting points of oils are less than 100°C and most are in fact liquid at body temperature.

Common oils include extracted and distilled oils from nuts and seeds, for example safflower, perrila, millet, niger, Ucuúba, sesame, cimbopogon, mustard, canola, corn, caraway, soybean, sunflower, garlic, peanut, pumpkin seed, olive, almond, macadamia, palm, walnut, pistachio, coconut, evening primrose seed, black currant seed, rosemary, borage seed or flax seed oils, and from fish and phytoplankton, for example shark, cod, mackerel, sardine, salmon, scrod, or halibut oils, or from oleagenous microorganisms directly. Also included are tocols (comprising the whole family of tocopherols and

tocotrienols, including the acetate esters), certain terpenoids comprising Vitamin A (also called retinol), retinoids, menaquinones such as Coenzyme Q, carotenoids such as carotenes, lycopene, and the related xanthophylls such as lutein, lutein esters, astaxanthin, canthaxanthin and zeaxanthin, Vitamin D, Vitamin K, vitamers of these Vitamin oils and their precursors, and glycerides high in PUFAs such as triglycerides containing esterified docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Also contemplated as oils are essential oils. These are often complex mixtures useful in enhancing bioavailability, and include extracts of (as in U.S. Patent No. 5,716,928) allspice berry, fennel, amber essence, anise seed, arnica, balsam of Peru, basil, bay leaf, parsley, peanut, benzoin gum, bergamot, rosewood, rosemary, rosehip, cajeput, marigold, turmeric, camphor, caraway, cardamom, carrot, cedarwood, celery, chamomile, cinnamon, citronella, palm kernels, avocado, macadamia, sage, clove, coriander, cumin, cypress, eucalyptus, aloe, fennel, fir, frankincense, garlic, geranium, rose, ginger, lime, grapefruit, orange, hyssop, jasmine, jojoba, juniper, lavender, lemon, lemongrass, marjoram, mugwort, watercress, mullen, myrrh, bigarde neroli, nutmeg, bitter orange, oregano, patchouly, pennyroyal, primrose, retinols, papaya, red pepper, black pepper, baccharis (Vassoura Oil), peppermint, poppyseed, petitegrain, pine, spruce, poke root, rosemary, sandalwood, sassafras, spearmint, spikenard, hemlock, tangerine, tea tree, thyme, vanilla, banana, coconut, vetivert, wintergreen, witch hazel, ylang ylang extract, or synthetic analogs.

Colloidal System: As used herein, this term refers to a system containing two or more immiscible phases, at least one of which takes the form of a particle or droplet and is termed a "dispersed" phase, and one phase is a liquid or a solution and is termed a "continuous" phase. As used herein, "colloidal systems" are limited to those wherein the particles are larger than simple molecular or micellar solutes but are small enough that they remain suspended in a fluid medium without settling to the bottom. The vapor pressure of a liquid or solution containing a colloid is typically not influenced by the colloidal particles or droplets in suspension. Nor are other colligative properties affected, for example osmolarity. A review of the prior art of colloidal systems is provided in Zografi, G. et al. 1990 "Disperse systems" in (Gennaro, A.R. and T. Medwick, eds.) *Remington's Pharmaceutical Sciences*, Philadelphia, PA. Emulsions, nanoemulsions, miniemulsions, and liposomes are examples of colloidal suspensions. Oil-in-water (o/w), water-in-oil (w/o), solid-in-oil, solid-in-water, and oil-in-solid colloidal systems are

known in the art. Colloidal systems are most preferably stabilized with surfactants. Aspects of the invention also include precursors of colloidal systems, for example, SEDDS (self-emulsifying drug delivery systems), wherein the oily phase containing a therapeutic agent is administered as a preconcentrate, typically with surfactant(s), sometimes with solvents, but absent water.

Multiphase System: As used herein, this term refers to a system where one or more phases is (are) dispersed throughout another phase, which is usually referred to as the continuous phase, or a precursor thereof. Complex emulsions, microemulsions and other multiphasic nanoparticulates, including liposomes, niosomes and crystalline suspensions in oil-in-water emulsions, are examples of multiphasic systems. These systems may be lyophilic or lyophobic. Biphasic systems are a subcategory of multiphasic systems.

Emulsion: A colloidal dispersion of two immiscible or poorly miscible liquid phases, such as oil and water, in the form of droplets. The internal phase is also termed the dispersed phase and the external phase is termed the continuous phase. The mean diameter of the dispersed phase, in general, is between about 0.2 and about 50.0 microns (μm), as is commonly measured by particle sizing methods, and the particles range broadly in size. Emulsions in which the dispersed phase and continuous phase have different refractive indexes are typically optically opaque. Emulsions in which the refractive indexes of the two phases are similar may be clear or translucent, and hence optical appearance is not a defining characteristic. Emulsions possess a finite or limited stability over time, and can be stabilized for days or weeks, sometimes for months, by the incorporation of surfactants and by viscosity modifiers.

Microemulsion: A thermodynamically stable, isotropically clear mixture of two immiscible liquids, stabilized by a relatively high concentration of surfactant molecules. Microemulsions have an apparent mean droplet diameter of less than about 200 nm, in general from about 10 to about 100 nm, and are typically self-assembling, or may be assembled using heat and/or solvents. Typically, microemulsions are more easily established with a co-surfactant. Microemulsions exist only within defined ratios of water, amphiphile and oil, as may be determined with a phase diagram for the system at a given temperature. Therefore, o/w microemulsions are inherently unstable when diluted with water. A class of microemulsions known as "swollen micelles" constitutes a system of special interest for drug delivery. Microemulsions of non-volatile oils decrease the

vapor pressure of the continuous phase, and conversely, microemulsions of volatile oils may increase the vapor pressure of the continuous phase, a change that may involve substantial departures from ideal solute behavior, where ideality is taken as an approximately linear relationship between solute concentration and vapor pressure.

Tocan Microemulsion: A thermodynamically stable, isotropically clear mixture of two immiscible liquids, one of which contains a tocan oil or tocan surfactant, stabilized by a relatively high concentration of surfactant molecules. Tocan microemulsions have an apparent mean droplet diameter of less than about 200 nm, in general from about 10 to about 120 nm. Once manufactured, however, they are filter sterilizable and are highly stable in a defined temperature range. Typically, tocan microemulsions are more easily established with a co-surfactant. A class of tocan microemulsions known as "swollen micelles" constitutes a system of special interest for drug delivery.

Nanoemulsion: Nanoemulsions, sometimes termed miniemulsions, are colloidal systems distinct from microemulsions and emulsions. As used herein, this term refers to those systems having a mean particle size that is less than 200 nm (as Gaussian volumetric mean), preferably less than 120 nm, and most preferably from about 10 to 100 nm, and not displaying apparent growth in particle size as measured by a lack of increase in size of greater than 15% in Gaussian volumetric mean by photon correlation spectroscopy when incubated at 25°C under controlled conditions for at least 30 days, preferably for 6 months, and most preferentially for up to 2 years. Some of these vehicles are isotropically clear and display high levels of drug loading (a relative property that must be determined independently for each drug). Some are translucent or hazy. Some nanoemulsions are self-assembling, but others may require heat, solvent, and/or increased shear to assemble due to the high viscosity of certain nutrient oils. Operationally, nanoemulsions, however formed, share the common property of being terminally filter sterilizable, typically by passage through a compatible filter membrane of a pore size not to exceed 0.2 microns. And unlike o/w microemulsions, o/w nanoemulsions are robust when diluted with aqueous IV solutions, an important property when used for drug delivery. In general, nanoemulsions are differentiated from microemulsions by their behavior upon dilution and from the broader category of emulsions by their size and stability. The relative insensitivity of the vapor pressure of the continuous phase to the presence of a nanoemulsion in suspension is a distinctive characteristic.

Tocan Nanoemulsions: Tocan nanoemulsion drug delivery vehicles contain a tocan surfactant or oil, optionally a cosurfactant, and an oil or oils and have a mean particle size that is less than 120 nm (as Gaussian volumetric mean), preferentially less than 100 nm, and do not display apparent growth in particle size (as measured by a lack of increase in size of greater than 15% in mean diameter by photon correlation spectroscopy) when incubated at 25°C under controlled conditions for at least 30 days, preferentially for 6 months, and most preferentially for up to 2 years. Many of these vehicles are optically translucent or clear, but display high levels of drug loading (a relative property that must be determined independently for each drug). Some tocan nanoemulsions are self-assembling, but others may require heat, solvent, and/or increased shear to formulate due to the high viscosity of certain tocan oils. Operationally, tocan nanoemulsions share the common property of being terminally filter sterilizable, typically by passage through a compatible filter membrane of pore size 0.2 microns. Tocol oil nanoemulsions are further characterized by modest or negligible effect on the vapor pressure of the continuous phase.

Liposome: A lipid bilayer vesicle formed spontaneously upon dispersion of certain lipids, most commonly phospholipids, in water. "Liposome" is also defined as a vesicular structure consisting of hydrated bilayers.

Tocan Liposome: A bilayer vesicle formed spontaneously upon dispersion of certain highly polar tocans in water. "Liposome" is also defined as a vesicular structure consisting of hydrated bilayers.

Niosome: By analogy to a liposome, a niosome is a nonionic surfactant vesicle. Classes of commonly used non-ionic surfactants include polyglycerol alkylethers, glucosyl dialkylethers, crown ethers and polyoxyethylene alkyl ethers and esters.

Tocan Niosome: By analogy to a liposome, a tocan niosome is a nonionic tocan surfactant vesicle.

Micelle: These are organized dynamic molecular aggregates of one or more surfactants that exist only at a concentration above the critical micellar concentration (CMC) in water or buffer. The CMC is an individual characteristic of each surfactant. These molecular aggregates typically have a nominal diameter of 2 to 6 nm, and perhaps 15 or 20 nm in some systems. Micellar solutions of surfactants cause departures from non-ideality of the vapor pressure of the continuous phase and have other properties not characteristic of colloids.

Self-Emulsifying Drug Delivery Systems (SEDDS): With reference to a phase diagram, certain mixtures of oil(s) and non-ionic surfactant(s) will form clear and isotropic solutions that then spontaneously emulsify when mixed with water. These mixtures, when comprising drug as well as oil and surfactant, are known as self-emulsifying drug delivery systems (SEDDS). Optionally, they may also contain solvents and other excipients. SEDDS and the related SMEDDS (self-microemulsifying drug delivery systems) have successfully been used to improve lipophilic drug dissolution and oral absorption.

Tocan Self-Emulsifying Drug Delivery Systems (TSEDDS): With reference to a phase diagram, certain mixtures of oil(s) and tocan surfactant(s) will form clear and isotropic solutions that then spontaneously emulsify when mixed with water. These mixtures, when comprising drug as well as oil and surfactant, are known as self-emulsifying drug delivery systems (SEDDS). Optionally, they may also contain solvents and other excipients. TSEDDS and the related TSMEDDS (tocan self-microemulsifying drug delivery systems) are useful to improve lipophilic drug dissolution and oral absorption.

Biocompatible: Capable of performing functions within or upon a living organism in a manner that does not terminate or excessively disable the life of the organism, i.e., without *undue* toxicity or harmful physiological or pharmacological effects.

Prodrug: A prodrug is a chemical derivative of a therapeutic agent which, following administration, is cleaved or metabolized to release the therapeutic agent *in situ*.

Hydrocarbyl: By "hydrocarbyl" is meant moieties containing carbon and hydrogen atoms only, with the indicated number of carbon atoms. Hydrocarbyl groups may be straight-chain or branched-chain, aliphatic or aromatic, alkanes or alkenes. Unsaturated groups such as 1- and 2-butene and propargyl, including multiple unsaturated groups such as butadienyl and phenyl or polyphenyl, are included in this term.

In one aspect, the present invention provides emulsion or liposome particles coated with a "stealth coat" so as to evade the phagocytic cells of the reticuloendothelial cell system (RES), comprising the liver, lungs, spleen and bone marrow. In one embodiment, particles that target particular cells where the therapeutic agent is needed, for example cancer cells.

A variety of polyoxyethylated derivatives have been used to form the "stealth coat" around the particles or liposomes. Examples of these agents include POLOXAMERS (also termed Pluronics®), which are block co-polymers of polyoxyethylene and polyoxypropylene, and pegylated surfactants such as Tween 80 and pegylated phospholipids. Particles coated with these molecules have been somewhat successful in evading the RES, but because of inherent toxicity or expense, have been less than satisfactory in clinical use. Furthermore, their corrosive detergency has rendered these compounds of little use in cosmetic applications.

Tocopherol, because of its gentle ability to stabilize biological membranes, is an excellent starting material to form a "stealth coat" surfactant for drugs and cosmetics. However, the chemical reactivity of the phenolic hydroxyl of tocopherol is limited and the ability to synthesize and screen tocol conjugates is substantially hampered by the strong reactants required to form a bond with the hydroxyl. Frequently, unacceptable side reactions occur or no reaction at all.

A related compound, 8-carboxytocol, which has been described in the literature, has been overlooked as a platform for the formation of prodrug and surfactant conjugates. This compound possesses excellent reactivity and versatility for synthesis of complex derivatives. The membrane insertion and stabilizing properties of this compound are expected to be retained and also expected to be biodegradable, albeit more slowly than esters or phosphate diesters. Furthermore, carboxytocans that retain the 6-hydroxyl were expected to be effective antioxidants.

The present invention provides benzopyran derivatives, including carboxytocans (and other tocans) as a platform for covalently coupling biomolecules to benzopyrans. In one embodiment, "stealth coat" surfactants comprising a hydrophilic "head" and a phytyl tail are provided. The very "greasy" phytyl or phytotrienyl tail can be used to position correspondingly large hydrophilic heads at a lipid/water interface. Whereas stearylamine has a molecular weight of 270 daltons, the analogous carboxytocan has a molecular weight of about 450 daltons, a 65% increase. Betaine, a good example of a corrosive and toxic detergent, has a CMC of about 0.0006 M, the CMC of TPGS is 0.0001 M, making TPGS a better detergent by a factor of six at low concentrations while gentle on cells. The large hydrophilic heads can also be used to prevent the interaction of the lipid droplets with phagocytic cells by "steric hindrance" and by binding of a layer of water to the particle through hydrogen bonds. In one embodiment, the benzopyran derivative is a

carboxytocan polyglutamate. Surfactants of this type are hydrophilic with high HLB values and are effective by steric hindrances to coalescence and by electrostatic repulsion in stabilizing emulsions during storage. These derivatives have excellent detergency but are gentle on biological membranes.

Polyglutamate is an effective "stealth coat." *Bacillus anthracis*, a highly virulent bacterium which multiplies unchecked in the blood stream of mammals, including man, uses a capsule of polyglutamate (gamma-linked) to avoid triggering an immune response and to avoid phagocytosis. Thus, an artificial particle encapsulated in a polyglutamate coat could also evade the immune system, leading to prolonged circulation in the bloodstream, a highly desirable outcome. In the invention, polyglutamate is covalently coupled to an appropriately substituted benzopyrans via mixed anhydride chemistry.

The primary advantage of selected carboxytocans as a starting material for formation of tocan derivatives is the highly chemically reactive character of the carboxyl, particularly for nucleophilic substitutions. Conceptually, this is a major advance over prior art, wherein substituent molecules are coupled to Vitamin E via a linker. This is because of the limited reactivity and strong reaction conditions required to render reactive the phenolic hydroxyl of tocols, chemistry which is known to be difficult or of limited versatility because of the relatively poor nucleophilicity of the phenolic hydroxyl and its rapid oxidation to yield undesirable chromogenic quinones (Skinner, W.A. and R.M. Parkhurst, 1970, "Reaction products of tocopherols" *Lipids* 6:240-44).

Generally, the addition of the biomolecule or targeting agent (see, e.g., R_5 in FIGURES 1-4 and 9-11) is the last step of any synthesis. The first steps involve formation of the tocan and functional group (carbonyl or carboxyl). This is preferentially done directly from a preferred starting material, often a natural product, so that the stereochemistry of the product can be preserved. For example, Vitamin E or Trolox is deoxygenated at the 6-position and then brominated so as to undergo Grignard mediated carboxylation at the 6-position to form 6-tocopherol carboxylate or 2,5,7,8-tetramethylchroman-2,6-dicarboxylic acid, respectively. Alternatively, δ-tocotrienol may be brominated directly at any of the open positions on the chroman ring, and carboxylated by metallation to yield mixtures of 5-, 7-, and 5,7-d-δ-tocotriene carboxylate. The 5-position is most reactive, comprising as much as 80% of the product. Alternatively, the 6'-O-tocol acetic acid ether can be formed by reacting a tocol with ethylbromoacetate and sodium ethoxide to alkylate the phenolic hydroxyl. Longer alkyl substituents may be

introduced by analogous chemistry. In another embodiment, a glycine linker can be introduced to form a tocoglycinate by first protecting the amine of the glycine with FMOC (9-fluorenylmethoxy-carbonyl) or t-BOC (tert-butoxy-carbonyl) and then activating the glycinate carboxyl with thionyl chloride to form the acid chloride. This intermediate will then participate in the nucleophilic attack on the phenolic hydroxyl of the tocol when mixed in the presence of TEA or other basic amine and a catalytic amount of DMF in anhydrous methylene chloride or THF.

Following introduction of the carbonyl, the R_5 group is then added to form the desired derivative. The chemistry of carboxytocan derivatives thus formed may preferentially include "amide" (also called "peptide") bonds of the form -NH-CO-. The amide bonds are preferentially formed by mixed anhydride chemistry, or may be formed with carbodiimides or phosgene as a condensing agent. The preferred amide bonds are biodegradable, but are more slowly degraded than ester, sulfhydryl, or diphosphoester bonds, and for that reason have surprising advantages as surfactants. However, ester bonds from the carbonyl are also suitable.

Regarding the chemistry of the carboxytocan conjugates, the chemical bond is preferably a "zero linker" amide bond. The active functional group is the 5, 6, 7, or 8 carboxyl on the benzopyran ring. However, by attaching bifunctional or multifunctional linkers such as glycine, glutamate, aspartic acid, cysteine, lysine, arginine, or even succinate to the carboxyl, additional functional reactivity is obtained. Both homomultifunctional and heteromultifunctional linkers are provided.

During coupling of the R_5 group to the benzopyran, delicate substituents must be protected from chemical degradation with "protective groups," which are subsequently cleaved off under mild conditions. For this reason, strong reaction conditions are impractical, a factor that rules out many common synthetic pathways. Mild or highly specific chemical synthetic routes that are practical often lack the yields required to be commercially desirable. Because of these limitations, the use of tocopherol as starting material for preparation of drug conjugates has been limited and highly specialized. No facile approach to tocopherol-drug conjugation has emerged despite 50 years of research. Given these requirements, the mixed anhydride chemistry of amide bonds for conjugation has clear advantages in terms of specificity, cost, and yield.

Carboxytocans as surfactants, for example as soaps, detergents, and cosmetics, will have good environmental compatibility, be biodegradable, be gentle on the skin or

mucosa, active in hard water, will absorb UV radiation, will adhere to skin, hair or fiber, and will be readily manufacturable. These are requirements that are advantages provided by the present invention.

The solvent and surfactant properties of carboxytocans, and their derivatives, for cosmetics and medicament delivery vehicles has not previously been evaluated. Carboxytocans can serve as biological detergents or as depot storage forms for the sustained-release delivery of drugs. Other multiphase systems include liquid crystalline structures such as liposomes, which are also suitable for drug delivery. In contrast, the relatively nonpolar tocopherols have proved rather poor in forming liposomes, and only 20 mol % tocopherol could be incorporated into liposomes made from phospholipids (Fukuzawa, K. et al., 1992, "Location and dynamics of α-tocopherol in model phospholipid membranes with different charges" *Chem. Phys. Lipids* 63:69-75) with little surface association of the phenolic hydroxyl. Carboxytocans are particularly useful in forming anionic surfactants and anionic liposomes. Similarly, ionic nanoparticles or nanocrystals are readily envisaged.

In another aspect, the invention provides compositions in which a pharmaceutical, nutriceutical, cosmeceutical, vitamin, foodstuff, antigen, catalyst, cell, nanoparticle, oligonucleotide, gene, extract, cosmetic or fiber is solubilized, protected or dispersed in a solution, particle, emulsion, microemulsion, nanoemulsion, liposome, niosome, molecular matrix or coating that include the benzopyran derivatives of the invention, optionally with other oils, co-solvents, surfactants and co-surfactants. Also described are specific examples of "zero linker" and linker-based carboxytocans and carboxybenzopyrans, and their derivatives, and the uses of carboxytocans as excipients, surfactants, as prodrugs, and as novel therapeutics, in creams, lotions, soaps, cosmetics, sunscreens, eyedrops, foodstuffs, toothpaste, detergents, emulsions, microemulsions, liposomes, capsules, nanosuspensions, nutriceuticals and injectables. The compositions may be administered orally, topically, or by other nonparenteral routes.

Regarding compositions containing the benzopyran derivatives of the present invention, many therapeutically useful drugs or other compounds are insoluble in water or poorly soluble, and must be administered in the form of an emulsion, microemulsion, or pre-emulsion concentrate, or as a micellar solution or liposome. By modifying the surface property of the emulsion droplet or liposome, the present invention provides a targeted therapeutic agent that can be direct to the desired cell. These tocan derivatives

can be used to modify the surface properties of emulsion droplets or liposomes so as to produce "stealth" or targeted medicament delivery formulations.

In another embodiment, the composition of the invention is a liposome that includes a tocan or derivative selected for its chemical stability and its ability to form bilayers alone and in mixtures with phospholipids and cholesterol or phytosterols. The tocans of the present invention form liposomes with novel surface properties.

In a further embodiment, targeted benzopyran derivatives are provided. The hydrophilic substituent of the tocan surfactant is selected from a list of compounds, such as folate, that are preferentially bound, adhere to, or are taken up by particular types of cells. In this way, the contents of the drug delivery vehicle can be targeted to these cells. See, e.g., Reddy, J.A. and P.S. Low, 1998, "Folate-mediated targeting of therapeutic and imaging agents to cancers" *Crit. Rev. Therapeutic Drug Carrier Systems* 15(6):587-627.

The benzopyran-containing compositions of the invention can be in the form of an emulsion, microemulsion, micellar solution, liquid crystalline system, self-emulsifying drug delivery system, or a liposomal formulation for parenteral administration. As used herein the term, "parenteral administration" includes intravenous, pulmonary, intraocular, intrathecal, transmucosal, intratracheal, transdermal, subcutaneous, intraperitoneal or intramuscular administration. Oil-in-water, water-in-oil, and bicontinuous emulsions (Shinoda, K. et al., 1984, "Principle of attaining very large solubilization" *J. Phys. Chem.* 88:5126), nanoemulsions, microemulsions, as well as liposomes, soaps, and detergents are suitable forms of the invention.

The following examples are provide for the purpose of illustrating, not limiting, the invention.

EXAMPLES

Example 1

The Synthesis of a Representative Benzopyran Derivative: 6'-O-d-δ-Tocopherol Acetic Acid Ether

In this example, the synthesis of a representative benzopyran derivative of the invention, a tocopherol acetic acid ether, is described.

d-δ-Tocopherol was purchased from Sigma Chemicals (St. Louis, MO). The synthetic scheme relies on ethylbromoacetate and sodium ethoxide to alkylate the phenolic hydroxyl of the 6-benzopyranol, introducing a carboxyl substituent as shown.

Stoichiometry for the reaction was based on a 10 gm batch size.

Compound	MW	moles	grams	mL	Density
d-δ-tocopherol	402.65	0.02	10		
ethyl bromoacetate	167	0.04	6.2	4.1	1.506
ethanol				50	
sodium ethoxide	68	0.02	1.7		
Theoretical Yield	12.1				-.

A solution of d-δ-tocopherol (10 g) in 50 mL of ethanol (absolute) was treated with 1.4 g of sodium ethoxide at ambient temperature and stirred for 15 minutes. There was an immediate color change to brown. After fifteen minutes, during which time all of the sodium ethoxide had dissolved, ethyl bromoacetate (3.5 mL) was added and the reaction stirred at ambient and monitored by TLC/NMR. A precipitate formed at once. TLC (95:5 hexane ethyl acetate) after four hours indicated a new and faster moving spot but also some unreacted starting material. The mixture was heated to reflux to drive the reaction to completion. After three hours, TLC (95:5 hexane ethyl acetate) indicated there was not much change. An additional 1.75 mL of ethyl bromoacetate was added and the reaction was stirred overnight at ambient.

TLC (95:5 hexane ethyl acetate) indicated no change. An additional 0.85 g of sodium ethoxide was added and stirring continued at ambient temperature. Two hours later, TLC (95:5 hexane ethyl acetate) indicated that the d-δ-tocopherol was virtually gone. There was a major product spot and two minor spots: δ-d-tocopherol and a faster moving spot that was in the d-δ-tocopherol originally. The reaction mixture was concentrated *in vacuo* and the residue was triturated with 200 mL of hexanes. The hexane solution was filtered and concentrated *in vacuo* to afford 12.4 g of crude product.

The light yellow oil was pumped on a Kugelrohr at 50°C and 0.2 Torr to remove solvent and excess ethyl bromoacetate. The NMR indicated that the desired product was present along with a trace of starting material (<5% based on integration) and some ethyl bromoacetate (ca. 5% based on integration). The crude product was loaded onto a 75S Biotage column in 50 mL of hexane and eluted with 4 L of 12:1 hexane ethyl acetate. Fraction two contained the fast moving spot, ethyl bromoacetate and some desired

product based on NMR and weighed 2.9 g. Fractions three through five were pure. They were combined and concentrated to afford 7.2 g of pure product based on the NMR. The yellow liquid could not move on a C18 column using acetonitrile water as a mobile phase but an MS was obtainable by direct injection. The M+1 = 489.4, M+23 = 511.4 and 2M+1 = 999.7 were all visible. Fractions 9-12 contained tocopherol. These fractions were discarded.

In a second step, the ethyl ester was removed to provide the carboxylic acid.

Compound	MW	moles	grams	mL	Density
ethyl 2-(tocopherolyl)acetate	488.74	0.01	7.2		
lithium hydroxide	23.95	0.02	0.42		
THF				25	
methanol				12.5	
water				5	

Ethyl 2-(tocopherolyl)acetate (7.2 g) was dissolved in 25 mL of THF and 12.5 mL of methanol and treated with 0.42 g of lithium hydroxide in water. The resulting mixture was stirred overnight at ambient temperature.

TLC (95:5 hexane:ethyl acetate) indicated that the ester had completely reacted, and that the reaction was complete. The reaction mixture was concentrated *in vacuo* and the residue triturated with hexanes, where it was completely soluble. The hexane solution was concentrated *in vacuo* to afford a creamy, slightly pink solid that was partitioned between 25 mL of 5% hydrochloric acid and 50 mL of ether. The aqueous layer was then extracted with 50 mL of ether and the combined ether extracts were dried over sodium sulfate and concentrated under vacuum first at aspirator pressure and finally at high vacuum to afford 5.2 g (81%) of an ivory, waxy solid. The NMR and IR were as expected for the desired product. Once again by HPLC, the product would not elute from the C18 column with acetonitrile water gradient, so a mass spectrogram was obtained by

direct injection. The M+1 = 461.3 and M+23 = 483.3 confirmed identity with the desired product, 2-(D-δ-tocopherolyl)-O-acetic acid ether. The product is illustrated in FIGURE 8.

The resulting product can be derivatized at the terminal carboxyl with appropriately reactive R_5 groups selected as hydrophilic substituents (i.e., not adherent to a C18 column by HPLC) to provide the benzopyran derivatives of the invention.

Example 2

The Synthesis of a Representative Benzopyran Derivative:

5-Carboxy-(d-δ-tocopheryl-6-ol)

In this example, the synthesis of a representative benzopyran derivative of the invention, a 5-carboxytocopherol, is described.

In the first step, the tocopherol was brominated. The reaction scheme is illustrated in FIGURE 12.

Compound	MW	moles	grams	mL	Density
d-δ-tocopherol	402.65	0.02	10		
bromine	159.8	0.02	4.0	1.3	3.119
carbon tetrachloride				50	

d-δ-Tocopherol (10 g) was dissolved in 25 mL of carbon tetrachloride, cooled to 0-5°C in an ice bath and treated with 1.3 mL of bromine in 25 mL of carbon tetrachloride over 30 minutes. The reaction was stored overnight at ambient temperature.

An aliquot was washed with saturated sodium bicarbonate, concentrated *in vacuo* and analyzed by NMR. Based on the doublet at 6.45 ppm for starting material and the product singlet at 6.7 ppm, the reaction was about 83% complete. An additional 0.26 mL of bromine was added dropwise at ambient temperature and the resulting mixture was stirred for three hours. An aliquot was washed with saturated sodium bicarbonate, concentrated *in vacuo* and analyzed by NMR. The reaction was complete based on the absence of the doublet at 6.45 ppm. The entire reaction mixture was partitioned between 50 mL of saturated sodium bicarbonate and 100 mL of ethyl acetate. The ethyl acetate layer was washed with 50 mL of saturated sodium thiosulfate, dried over sodium sulfate and concentrated *in vacuo* to afford 13.4 g of a yellow oil. This was dissolved in 20 mL of hexanes and poured onto a column of 60 g of silica gel dispersed in hexanes in a

sintered glass funnel. The column was eluted with 200 mL of hexanes (fraction one), then with 1 L of 95:5 hexanes ethyl acetate. Fractions 2 and 3 contained product by TLC. Fractions 4 and 5 were colored but nothing was visible by TLC (95:5 hexanes ethyl acetate). Fractions 2 and 3 were combined and concentrated to afford 10.8 g (90.3%) of 5-bromo-d-δ-tocopherol. The NMR indicated the desired product.

In a second step, the brominated tocopherol was converted to the carboxytocopherol. The reaction scheme is illustrated in FIGURE 13.

Compound	MW	moles	grams	mL	Density
5-bromo-d-δ-tocopherol	481.55	0.01	7		
butyl lithium 2.5 M in hexanes		0.03		12.2	
THF (anhydrous)				50	
carbon dioxide	44	0.07	3.2		
5% hydrochloric acid	36.4	0.06	2.2	44.4	

5-Bromo-d-δ-tocopherol (5 g) was dissolved in 25 mL of THF (anhydrous) and cooled to -60°C. Butyl lithium (2.5 M, 8.7 mL) was added dropwise over 10-15 minutes and the reaction was held at <-60°C for three hours. During the first half of the addition, the temperature evolved heat and had to be stopped repeatedly to hold the temperature below -60°C. After about one-half of the butyl lithium was added, the rate of addition could be increased greatly. The solution was clear yellow. After three hours at -60°C, an aliquot was quenched with 5% hydrochloride acid, sparged with carbon dioxide gas, and then extracted with ethyl acetate and concentrated *in vacuo*. Based on the presence of a doublet at 6.45 ppm compared to the singlet at 6.7 ppm in the NMR, the metallation had proceeded to completion.

The NMR of this oil indicated that it was mostly bromide but the TLC in 95:5 hexanes ethyl acetate indicated a slower moving product (lower Rf than tocopherol itself). The product was chromatographed on a Biotage 40M using 95:5 hexanes ethyl

acetate to elute. Fractions 4-11 contained starting bromide based on TLC Rf. Fractions 15-16 contained the desired product. Fractions 17-21 appeared to be the pure product but NMR indicated that there was an impurity (valeric acid) at 2.4 ppm. The oil was heated on a Kugelrohr at 35°C and 0.2 torr and the impurity at 2.4 ppm decreased, but did not disappear. Increasing the temperature to 50°C distilled off the last of the valeric acid based on the NMR. The IR showed a strong carbonyl at 1643 cm-1 (salicylic acid has a carbonyl stretch of 1648 cm-1). There were 6 aromatic signals and 1 carbonyl by 13C NMR. LCMS by direct injection indicated an M+1 of 447 (+H), an M+23 of 469 (+Na) and an M+39 of 485 (+K), indicative for the desired product. The product is illustrated in FIGURE 13.

Example 3

The Synthesis of a Representative Benzopyran Derivative:

RRR-d-α-Tocopheryl-6-carboxylic acid

In this example, the synthesis of a representative benzopyran derivative of the invention, a 6-carboxytocopherol, is described.

Synthesis of RRR-d-α-6-tocopherol-carboxylate by modifications of the original route used to assemble α-tocopherol (Vitamin E), wherein the appropriate phenolic compound is condensed with phytol, is problematic because the chemistry would eventually prove difficult to scale (e.g., diazonium chemistry), the overall yield is low (primarily due to the purifications required), and the product may be a racemate.

Conversion of RRR-d-α-tocopherol to RRR-d-α-tocopherol-6-carboxylate is depicted in FIGURE 14. The advantage of this route is significant: all the necessary carbons and connectivity are present in the starting material (Vitamin E), which is abundant, inexpensive and readily available.

Deoxygenation of a phenol begins by activating the phenolic oxygen as some derivative, such as tosylate, isourea, dimethylthiocarbonate, or triflate, followed by reduction (Sebok, P.; Timar, T.; Eszenyi, T., 1994, *J. Org. Chem.* 59:6318-6321; Wang, F.; Chiba, K.; Tada, M., 1992, *J. Chem. Soc. Perkin Trans.* 1 1992:1897-1900; Saa, J.M.; Dopico, M.; Martorell, G.; Garcia-Raso, A., 1990, *J. Org. Chem.*, 55:991-995). Whether this would work with a benzopyran was unknown. The method chosen involved activation of the phenolic oxygen as a triflate and then either catalytic hydrogenation (Raney nickel) or chemical reduction ($NaBH_4$, $NiCl_2$). Tosylate activation is less expensive than triflate but triflate was more successful with the hindered phenol.

Reduction of Phenol Derivatives.

Derivative	Conditions	Reference	Observations
$-SO_2Tol$	H_2, Raney Ni NaOH, 5 atm OR $NaBH_4/NiCl_2$	3,4	economical and scalable
$-CNHNEt_2$	H_2/Pd on carbon EtOH, RT, 5 atm	3	three-step sequence
$-SCNMe_2$	Raney Ni EtOH, RT	3	four-step sequence
$-SO_2CF_3$	H_2/Pd on carbon $H_2/(Ph_3P)_2PdCl_2$/ Bu_3N/DMF	5	compatible with highly substituted phenols

Subsequently, the resulting hydrocarbon can be brominated (bromine, acetic acid). Because there is only one site on the aromatic ring that can react with bromine, the reaction is expected to be high yielding. The resulting bromide can then be converted to its corresponding Grignard reagent, which on treatment with carbon dioxide and then aqueous acidic workup should afford the desired product.

Example 4

Synthesis of 2,6 Dimethylbenzylcarboxylate

As a model synthesis for demonstration of a mixed anhydride route to carboxybenzopyran derivatives, synthesis of 2,6 dimethylbenzylcarboxy-glutamate was carried out via mixed anhydride chemistry. 2,6-Dimethyl benzoic acid was first activated by adding 1.00 equivalents of isobutyl chloroformate (IBCF) and N-methylmorpholine (NMM) in anhydrous tetrahydrofuran (THF) medium at -5°C. The reaction mixture was stirred at -5°C for 60 mins. The mixed anhydride was filtered to remove the N-methylmorpholine hydrochloride salt (NMM:HCl). The filtrate was added dropwise to a solution of 1.2 equivalents of H-Glu(Obzl)-OH in THF containing 1.2 equivalents of triethyl amine (TEA) at -5°C. The solution was left stirring overnight.

After completion of the reaction, the THF was removed in vacuum and a sticky product was obtained. The product was dissolved in dichloromethane (DCM) and washed with 2x0.1 N HCl, 2x $NaHCO_3$ (satd.), 1x NaCl (satd.). The resulting organic mixture was dried over $MgSO_4$ and, dried under vacuum to yield a white sticky crystalline solid, yield 94%. FTIR of amine, acid, aromatic (N-H, C=O, C-H) at 3067 and 3034, 1713, 2963 cm^{-1}, respectively.

Example 5

The Synthesis of a Representative Benzopyran Derivative:

6-O-d-δ-Tocopherol Acetic Acid Ether-Polyglutamate

In this example, the synthesis of a representative benzopyran derivative of the invention, 6-O-d-δ-tocopherol acetic acid ether-polyglutamate, is described.

The synthesis of 6-O-d-δ-tocopherol acetic acid ether -polyglutamate was carried out via mixed anhydride chemistry. 6'-O-d-δ-Tocopherol acetic acid ether (prepared as described in Example 1) was activated by adding 1.00 equivalents-of isobutyl chloroformate (IBCF) and N-methylmorpholine (NMM) in anhydrous tetrahydrofuran (THF) medium at -5°C. The reaction mixture was stirred at -5°C for 60 mins. The mixed anhydride was filtered to remove the N-methylmorpholine hydrochloride salt (NMM:HCl). The filtrate was added dropwise to a solution of 1.00 equivalence of poly (γ-Obzl)glutamate in THF containing 1.00 equivalence of triethylamine(TEA) at -5°C. The solution was left stirring overnight. After completion of the reaction, the THF was removed in vacuum and colorless oil was obtained. The product was dissolved in dichloromethane (DCM) and washed with 2x 0.1 NHCl, 2x $NaHCO_3$ (satd.), lx NaCl (satd.). The resulting organic mixture was dried over $MgSO_4$ and dried under vacuum to yield a colorless oily product, yield 91%. The deprotection of 6'-O-d-δ-tocopherol acetic acid ether -poly((γ-Obzl)) glutamate was carried by hydrogenation using Pd/C (10%) as catalyst, yield=55%, FT-IR of amide, acid, amide, aliphatic (N-H, C=O, C=O, C-H) are 3285, 1710, 1654, 2950 cm^{-1}, respectively.

Example 6

The Synthesis of a Representative Benzopyran Derivative:

6-O-d-δ-Tocopherol Acetic Acid Ether-Triglutamate

In this example, the synthesis of a representative benzopyran derivative of the invention, 6-O-d-δ-tocopherol acetic acid ether-triglutamate, is described.

The synthesis of 6-O-d-δ-tocopherol acetic acid ether-monoglutamate was carried out via mixed anhydride chemistry. 6-O-carboxymethyl-d-δ-tocopherol acetic acid ether (prepared as described in Example 1) was activated by adding 1.00 equivalents of isobutyl chloroformate (IBCF) and N-methylmorpholine (NMM) in anhydrous tetrahydrofuran (THF) medium at -5°C. The reaction mixture was stirred at -5°C for 60 mins. The mixed anhydride was filtered to remove the N-methylmorpholine hydrochloride salt (NMM:HCl). The filtrate was added dropwise to a solution of 1.2 equivalents of glutamic acid trimer (H-Glu(Obzl)-Glu(Obzl)-Glu(Obzl)) in THF containing 1.2 equivalents of triethyl amine(TEA) at -5°C. The solution was left stirring overnight. After completion of the reaction, the THF was removed in vacuum and colorless oil was obtained. The product was dissolved in dichloromethane (DCM) and washed with 2x 0.1 N HCl, 2x $NaHCO_3$ (satd.), lx NaCl (satd.). The resulting organic mixture was dried over $MgSO_4$ and dried under vacuum to yield a colorless oily product, yield 91%.

Example 7

The Synthesis of a Representative Benzopyran Derivative:

6-O-d-δ-Tocopherol Acetic Acid Ether-Monoglutamate

In this example, the synthesis of a representative benzopyran derivative of the invention, 6-O-d-δ-tocopherol acetic acid ether-monoglutamate, is described.

The synthesis of 6-O-d-δ-tocopherol acetic acid ether-monoglutamate was carried out via mixed anhydride chemistry. 6-O-d-δ-Tocopherol acetic acid ether (prepared as described in Example 1) was activated by adding 1.00 equivalents of isobutyl chloroformate (IBCF) and N-methylmorpholine (NMM) in anhydrous tetrahydrofuran (THF) medium at -5°C. The reaction mixture was stirred at -5°C for 64 mins. The mixed anhydride was filtered to remove the N-methylmorpholine hydrochloride salt (NMM:HCl). The filtrate was added dropwise to a solution of 1.2 equivalents of glutamic acid dibenzyl ester p-toluenesulfonate salt in THF containing 1.2 equivalents of triethylamine (TEA) at -5°C. The solution was left stirring overnight. After completion of the reaction, the THF was removed in vacuum and colorless oil was obtained. The product was dissolved in dichloromethane (DCM) and washed with 2x 0.1 N HCl, 2x $NaHCO_3$ (satd.), 1x NaCl (satd.). The resulting organic mixture was dried over $MgSO_4$ and dried under vacuum to yield a colorless oily product, yield 92%. The deprotection of 6'-O-d-δ-tocopherol acetic acid ether-monoglutamate dibenzyl ester was carried by

hydrogenation using Pd/C (10%) as catalyst, yield=73%, FT-IR of amide, acid, amide, aliphatic hydrogen (N-H, C=O, C=O, C-H) are 3285, 1710, 1654, 2950 cm^{-1}, respectively.

Example 8

The Synthesis of a Representative Benzopyran Derivative:

d-δ-Tocopherol-5-carboxylic Acid Taurine Amide

In this example, the synthesis of a representative benzopyran derivative of the invention, d-δ-tocopherol-5-carboxylic acid taurine amide, is described.

The synthesis of taurine amide of d-δ-tocopherol-5-carboxylic acid conjugate is carried out via mixed anhydride chemistry. 1.00 equivalent of d-δ-tocopherol-5-carboxylic acid is activated by adding 1.00 equivalents of isobutyl chloroformate (IBCF) and N-methylmorpholine (NMM) in 100 ml of anhydrous tetrahydrofuran (THF) medium at -5°C. The reaction mixture is stirred at -5°C for 60 mins. The mixed anhydride is filtered to remove the N-methylmorpholine hydrochloride salt (NMM:HCl). The filtrate is added dropwise to 1.00 equivalent solution of aminoethanesulfonic acid (Taurine, Sigma Chemicals) in THF containing 1.2 equivalents of triethylamine (TEA) at -5°C. The solution is left stirring overnight. After completion of the reaction, the THF was removed under vacuum and an oil is obtained. The product is dissolved in dichloromethane (DCM) and washed with 2x 0.1 N HCl, 2X $NaHCO_3$ (satd.), 2x NaCl (satd.). The resulting organic mixture is dried over $MgSO_4$ and then under vacuum to yield an oily product.

Example 9

The Synthesis of a Representative Benzopyran Derivative:

d-δ-Tocopherol-5-carboxylic Acid t-BOC-PEG Amide

In this example, the synthesis of a representative benzopyran derivative of the invention, d-δ-tocopherol-5-carboxylic acid t-BOC PEG amide, is described.

The synthesis of d-δ-tocopherol-5-carboxylic acid is carried out via mixed anhydride chemistry. 0.9 Equivalent of d-δ-tocopherol-5-carboxylic acid is activated by adding 0.9 equivalents of isobutyl chloroformate (IBCF) followed by addition of 0.9 equivalents of N-methylmorpholine (NMM) in 100 ml of anhydrous tetrahydrofuran (THF) medium at -5°C. The reaction mixture is stirred at -5°C for 60 mins. The mixed anhydride is filtered to remove the N-methylmorpholine hydrochloride salt (NMM:HCl). The filtrate is added dropwise to 1.00 equivalent solution of t-BOC-NH-PEG-NH_2

(Shearwater Polymers Inc., Los Angeles, CA) in THF containing 1.0 equivalents of triethylamine (TEA) at -5°C. The solution is left stirring overnight. After completion of the reaction, the THF was removed in vacuum and a transparent waxy product is obtained which is dried under high vacuum overnight. The t-boc-PEG-d-δ-tocopherol-5-carboxylic acid is washed with 3x50 ml diethyl ether and free flowing white conjugate product is obtained. This in turn is deprotected to release the pegylated carboxytocan which is a distant and novel relative of TPGS. Note the hydroxyl at the 6 position forms a functional antioxidant while the carboxyl at the 5 position permits derivatization for novel biological properties and surfactants.

Example 10

Surfactant Properties of Representative Benzopyran Derivatives

The mono- and tri-glutamyl benzopyran derivatives prepared as described in Examples 6 and 7 were tested for their properties as a surfactant by measuring surface tension at a standard concentration in comparison to other surfactants. The surface tension (γ_s) of a 0.1% solution in 20 mM phosphate buffer pH 7.4 was measured using a K12 Tensiometer (Kruss, Charlotte, NC) equipped with a Wilhelmy platinum plate.

Sample	Surface Tension (0.1% w/v, dyne/cm)
Water	71.9
Tococarboxy monoglutamate	41.0
Tococarboxy triglutamate	32.2
TPGS	34.5
Amisoft	24.8

For comparison, a pure non-ionic surfactant, TPGS (Eastman, Kingsport, TN), and a pure anionic surfactant, Amisoft (Ajinomoto, Tokyo, JP), were also tested at 0.1%

in water. The tococarboxy monoglutamate derivative was superior to TPGS (a nonionic surfactant sold commercially), and tococarboxy triglutamate derivative was comparable to TPGS.

Example 11

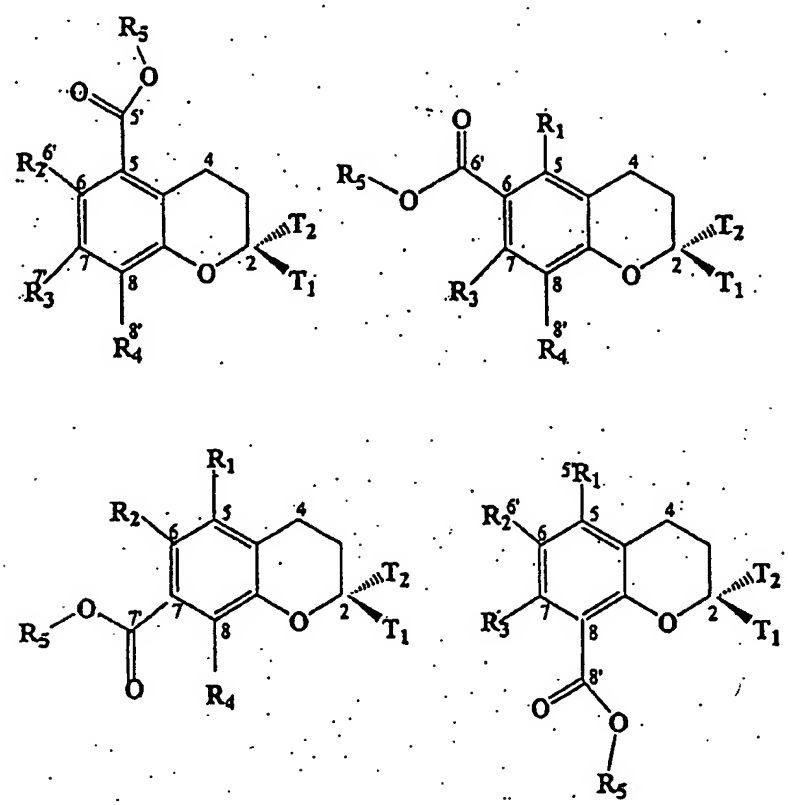
Oral Bioavailability

The benzopyran derivatives of the invention can be evaluated as bioavailability enhancers in an in vitro CACO-2 tissue culture model.

While the preferred embodiment of the invention has been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A compound having the structure:

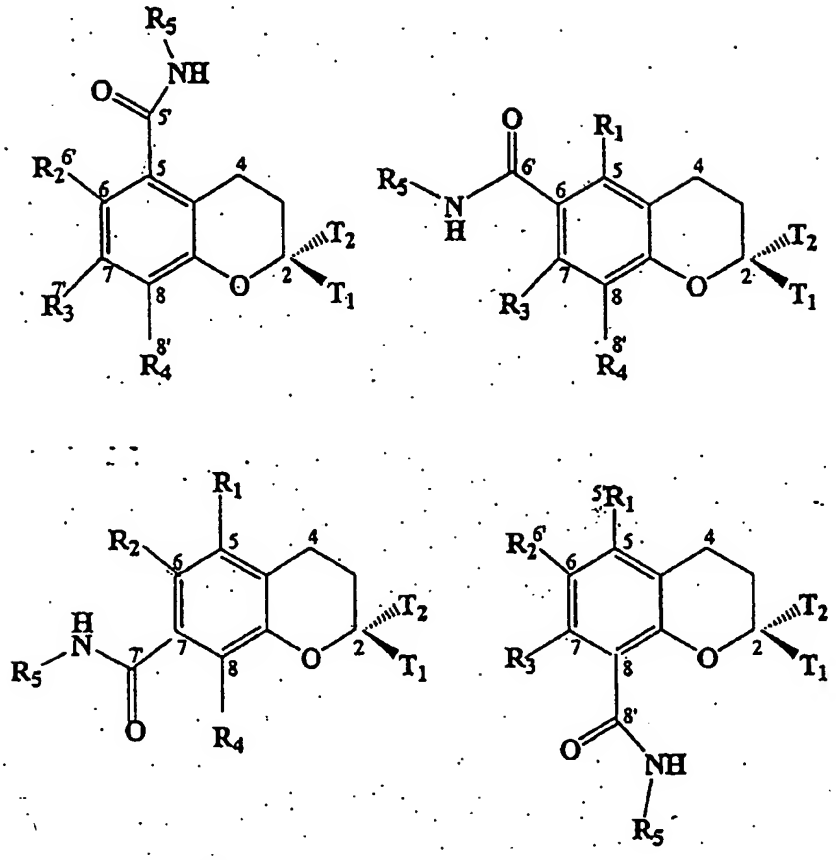


wherein R1, R2, R3, and R4 are selected from the group consisting of hydrogen, C1-C4 alkyl, carboxyl, and hydroxyl;

wherein R5 is a hydrophilic group that renders the compound no more than weakly bonded to a C8 column packing; and

wherein T1 and T2 are selected from the group consisting of hydrogen, methyl, ethyl, isoprenyl, terpenyl, phytyl, and trienyl.

2. A compound having the structure:

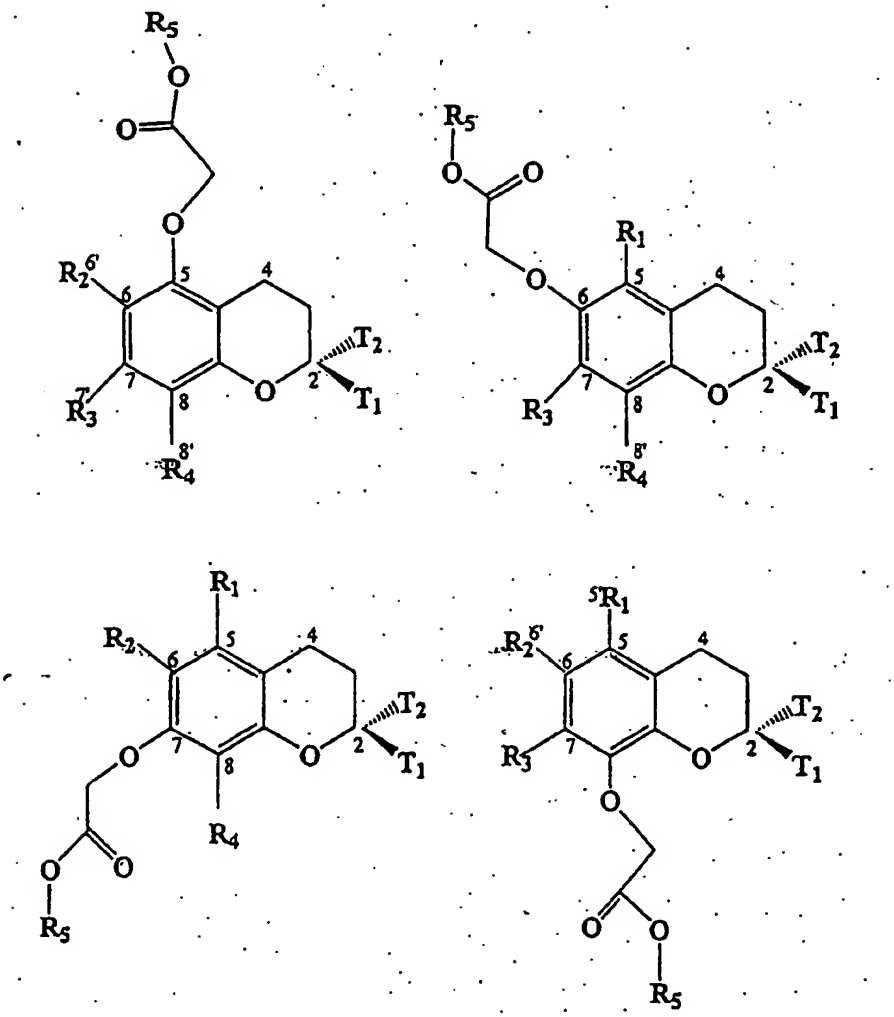


wherein R1, R2, R3, and R4 are selected from the group consisting of hydrogen, C1-C4 alkyl, carboxyl, and hydroxyl;

wherein R5 is a hydrophilic group that renders the compound no more than weakly bonded to a C8 column packing; and

wherein T1 and T2 are selected from the group consisting of hydrogen, methyl, ethyl, isoprenyl, terpenyl, phytyl, and trienyl.

3. A compound having the structure:

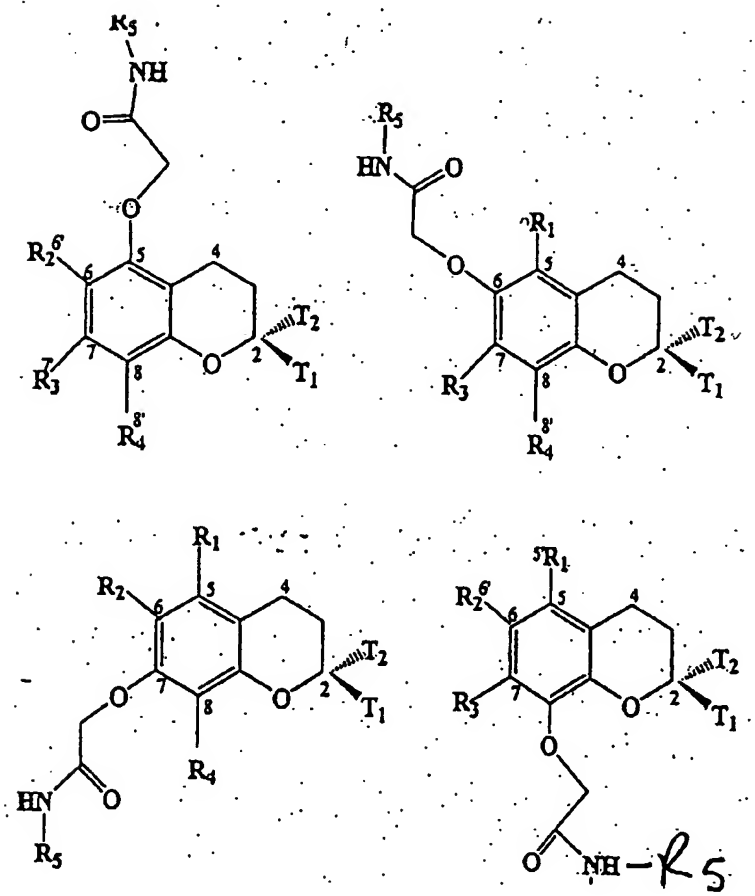


wherein R1, R2, R3, and R4 are selected from the group consisting of hydrogen, C1-C4 alkyl, carboxyl, and hydroxyl;

wherein R5 is a hydrophilic group that renders the compound no more than weakly bonded to a C8 column packing; and

wherein T1 and T2 are selected from the group consisting of hydrogen, methyl, ethyl, isoprenyl, terpenyl, phytyl, and trienyl.

4. A compound having the structure:

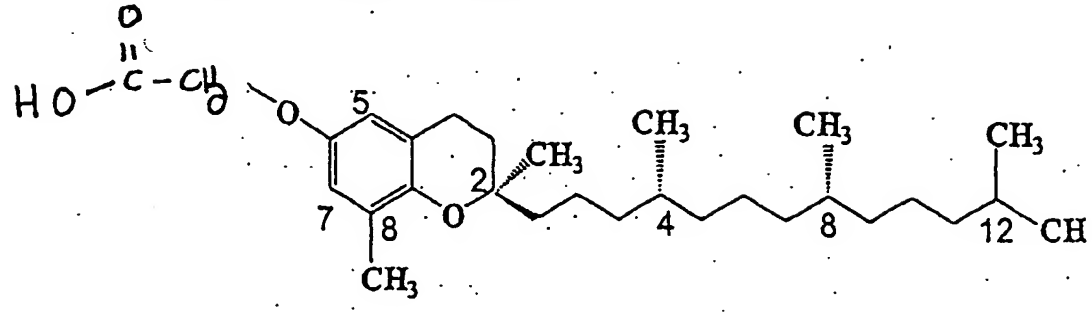


wherein R1, R2, R3, and R4 are selected from the group consisting of hydrogen, C1-C4 alkyl, carboxyl, and hydroxyl;

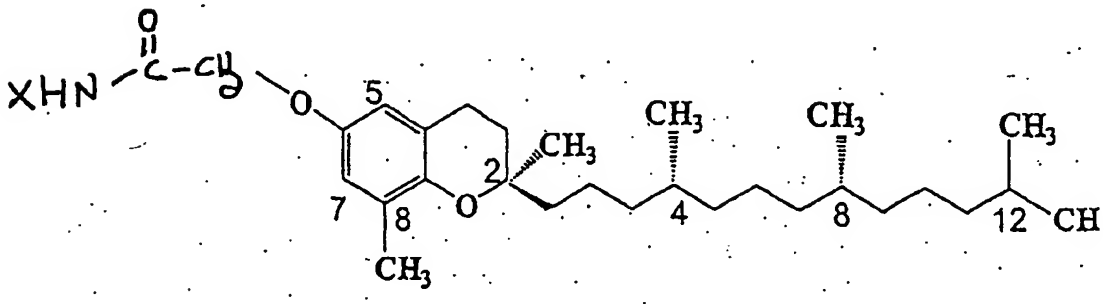
wherein R5 is a hydrophilic group that renders the compound no more than weakly bonded to a C8 column packing; and

wherein T1 and T2 are selected from the group consisting of hydrogen, methyl, ethyl, isoprenyl, terpenyl, phytyl, and trienyl..

5. A compound having the structure:



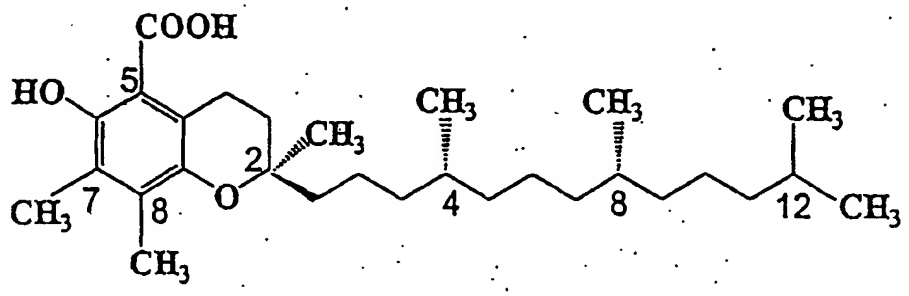
6. A compound having the structure:



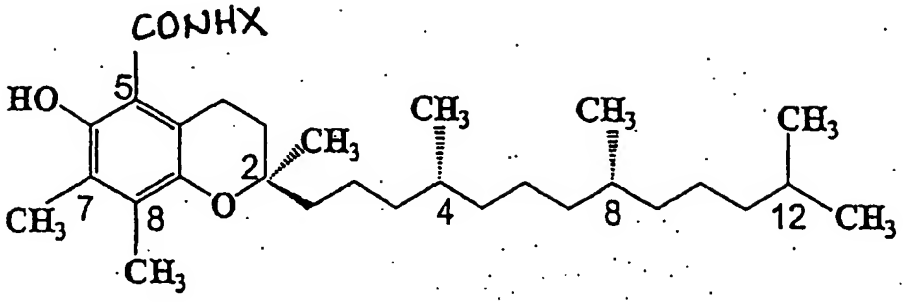
wherein NHX comprises an amino acid residue.

7. The compound of Claim 6, wherein the amino acid residue is selected from the group consisting of glutamic acid, triglutamic acid, and polyglutamic acid.

8. A compound having the structure:

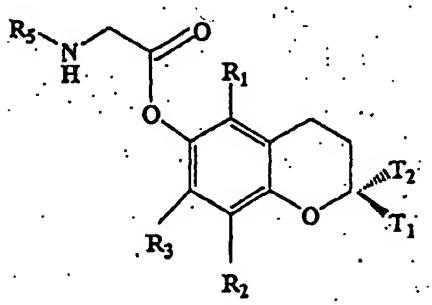


9. A compound having the structure:



wherein NHX comprises an amino acid residue.

10. A compound having the structure:

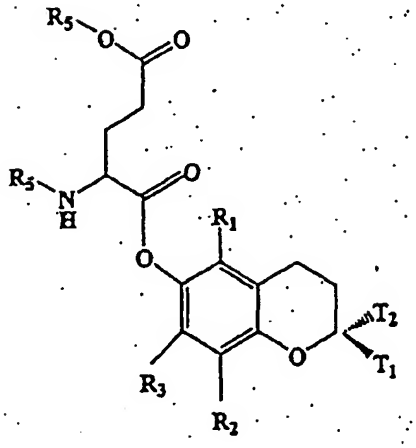


wherein R1, R2, and R3 are selected from the group consisting of hydrogen, C1-C4 alkyl, carboxyl, and hydroxyl;

wherein R5 is a hydrophilic group that renders the compound no more than weakly bonded to a C8 column packing; and

wherein T1 and T2 are selected from the group consisting of hydrogen, methyl, ethyl, isoprenyl, terpenyl, phytyl, and trienyl.

11. A compound having the structure:

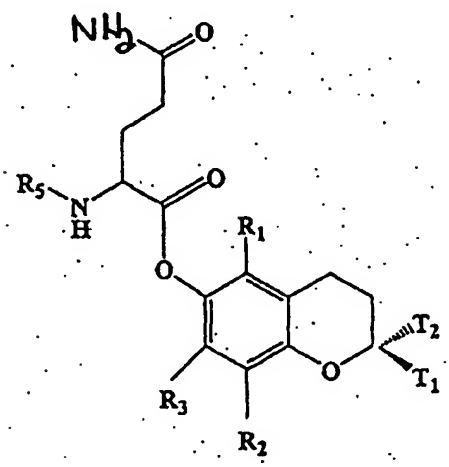


wherein R1, R2, and R3 are selected from the group consisting of hydrogen, C1-C4 alkyl, carboxyl, and hydroxyl;

wherein R5 is a hydrophilic group that renders the compound no more than weakly bonded to a C8 column packing; and

wherein T1 and T2 are selected from the group consisting of hydrogen, methyl, ethyl, isoprenyl, terpenyl, phytyl, and trienyl.

12. A compound having the structure:



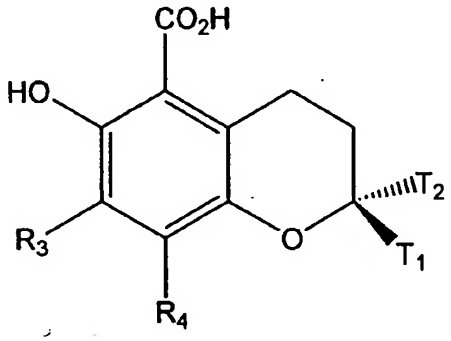
wherein R1, R2, and R3 are selected from the group consisting of hydrogen, C1-C4 alkyl, carboxyl, and hydroxyl;

wherein R5 is a hydrophilic group that renders the compound no more than weakly bonded to a C8 column packing; and

wherein T1 and T2 are selected from the group consisting of hydrogen, methyl, ethyl, isoprenyl, terpenyl, phytyl, and trienyl.

13. The compound of Claim 12, wherein R5 comprises an amino acid residue.

14. A compound having the structure:

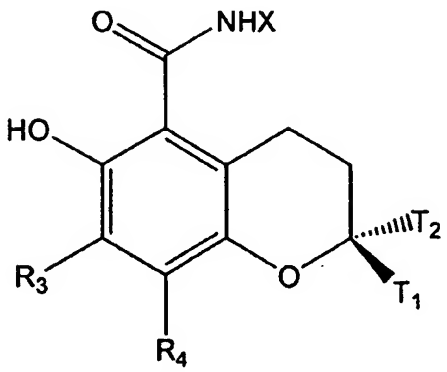


wherein R3 and R4 are selected from the group consisting of hydrogen, C1-C4 alkyl, carboxyl, and hydroxyl; and

wherein T1 and T2 are selected from the group consisting of hydrogen, methyl, ethyl, isoprenyl, terpenyl, phytyl, and trienyl.

15. The compound of Claim 14, wherein R3 is hydrogen, R4 is methyl, and T1 and T2 are selected from the group consisting of methyl and phytyl.

16. A compound having the structure:



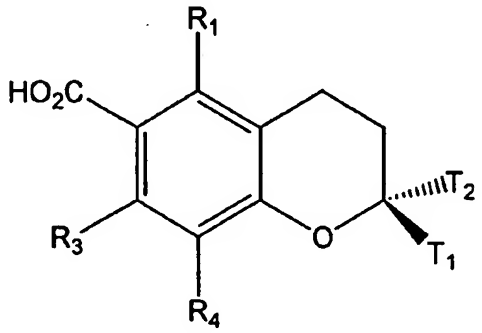
wherein R3 and R4 are selected from the group consisting of hydrogen, C1-C4 alkyl, carboxyl, and hydroxyl;

wherein NHX comprises an amino acid residue; and

wherein T1 and T2 are selected from the group consisting of hydrogen, methyl, ethyl, isoprenyl, terpenyl, phytyl, and trienyl.

17. The compound of Claim 16, wherein R3 is hydrogen, R4 is methyl, and T1 and T2 are selected from the group consisting of methyl and phytyl.

18. A compound having the structure:

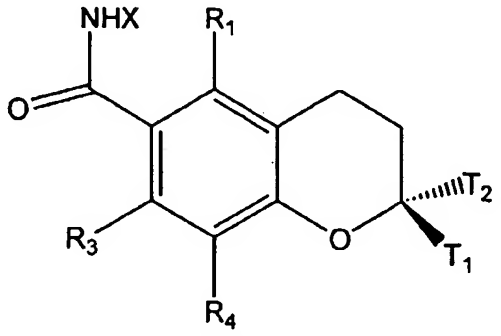


wherein R1, R3, and R4 are selected from the group consisting of hydrogen, C1-C4 alkyl, carboxyl, and hydroxyl; and

wherein T1 and T2 are selected from the group consisting of hydrogen, methyl, ethyl, isoprenyl, terpenyl, phytyl, and trienyl.

19. The compound of Claim 18, wherein R1, R3, and R4 are methyl, and T1 and T2 are selected from the group consisting of methyl and phytyl.

20. A compound having the structure:



wherein R1, R3, and R4 are selected from the group consisting of hydrogen, C1-C4 alkyl, carboxyl, and hydroxyl;

wherein NHX comprises an amino acid residue; and

wherein T1 and T2 are selected from the group consisting of hydrogen, methyl, ethyl, isoprenyl, terpenyl, phytyl, and trienyl.

21. The compound of Claim 20, wherein R1, R3, and R4 are methyl, and T1 and T2 are selected from the group consisting of methyl and phytyl.

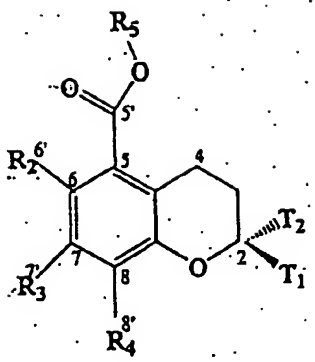


FIG. 1A

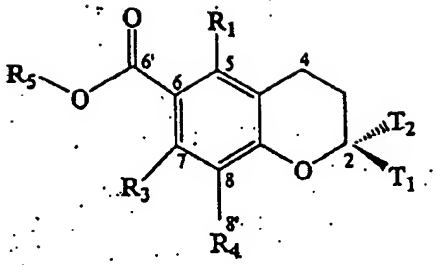


FIG. 1B

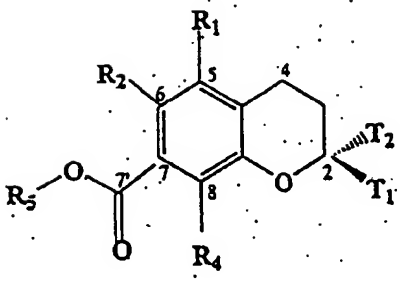


FIG. 1C

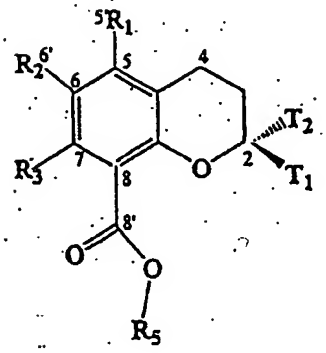


FIG. 1D

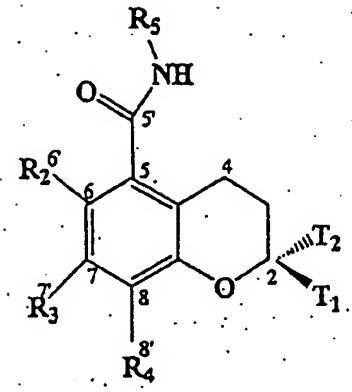


FIG. 2A

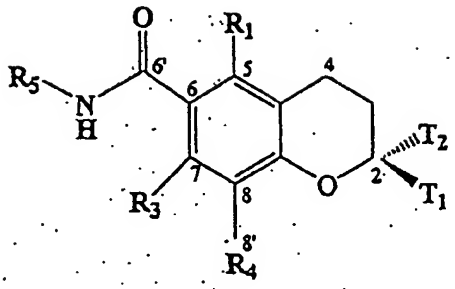


FIG. 2B

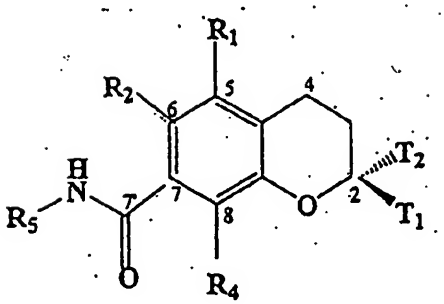


FIG. 2C

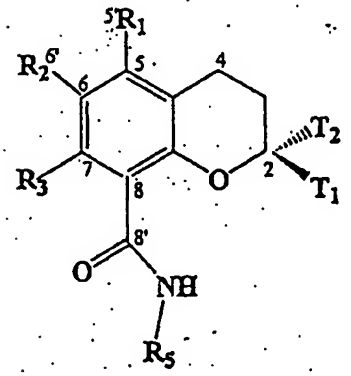


FIG. 2D

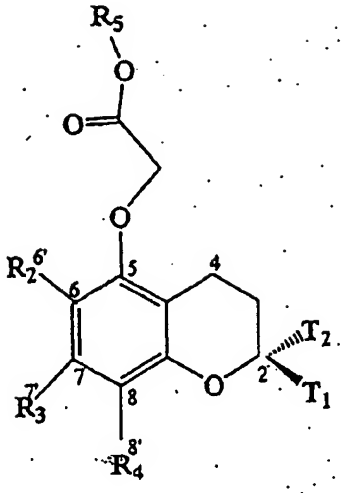


FIG. 3A

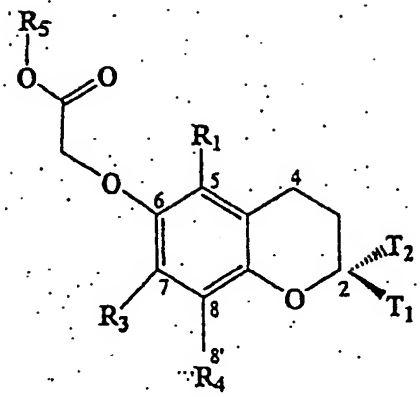


FIG. 3B

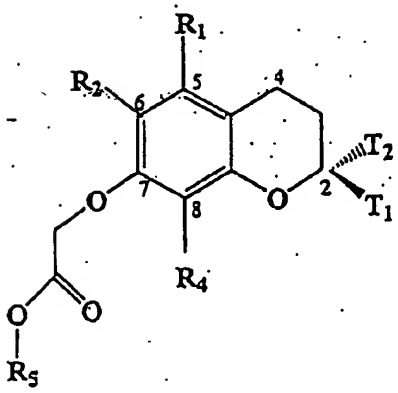


FIG. 3C

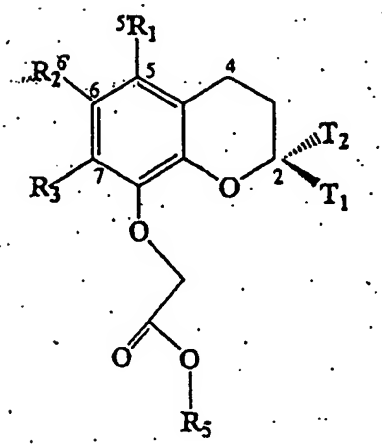


FIG. 3D

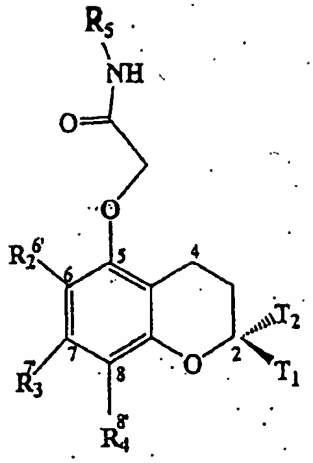


FIG. 4A

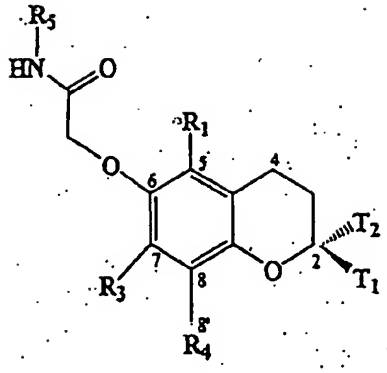


FIG. 4B

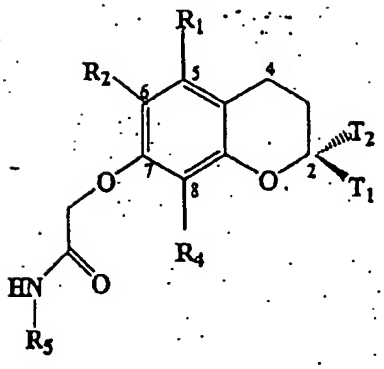


FIG. 4C

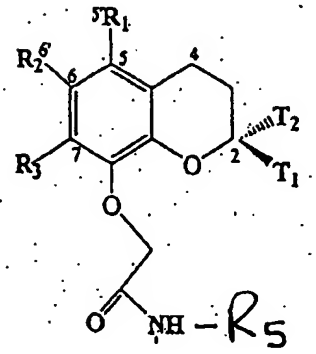


FIG. 4D

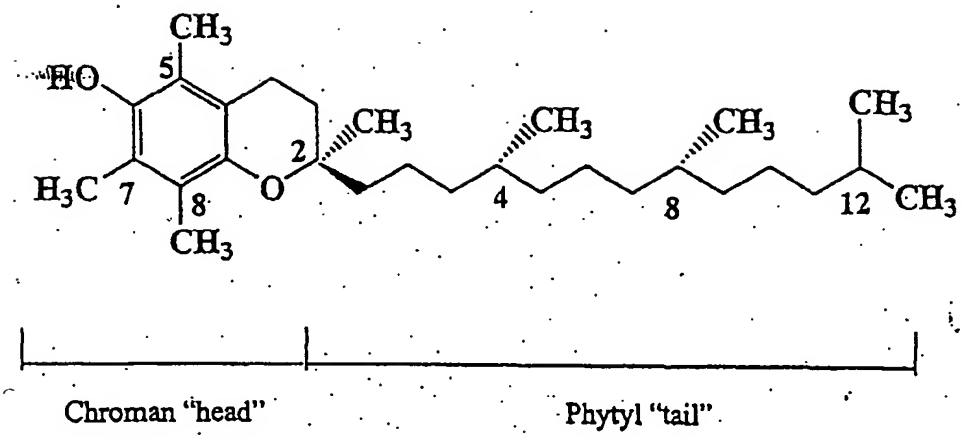

CH3
HO
5
CH3
CH3
CH3
CH3
2
H3C
7
8
O
4
8
12
CH3
CH3
Chroman "head"
Phytyl "tail"

FIG. 5

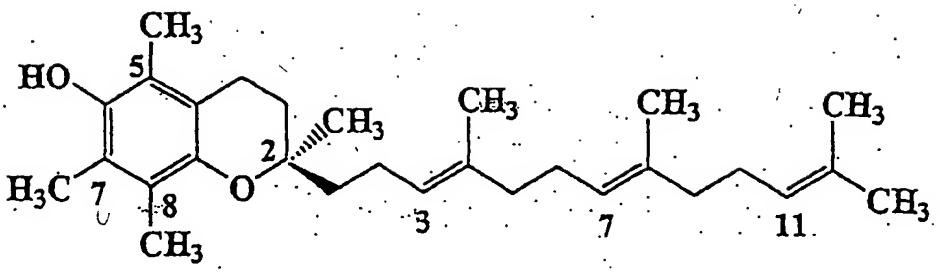


FIG. 6

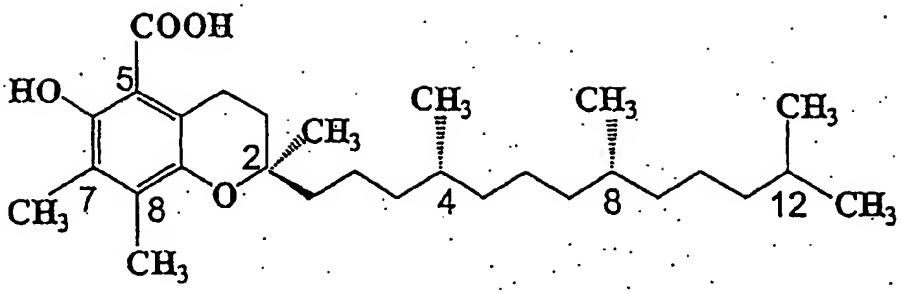


FIG. 7

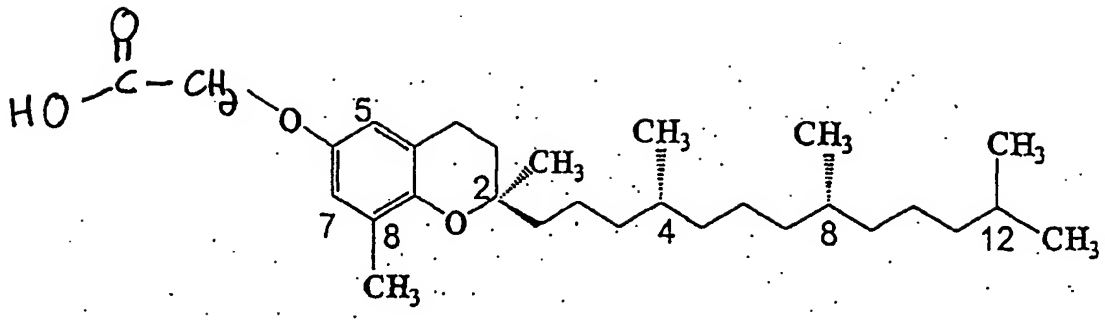


FIG. 8

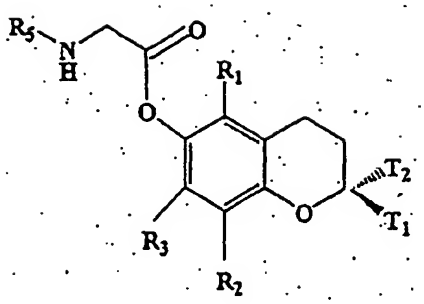


FIG. 9

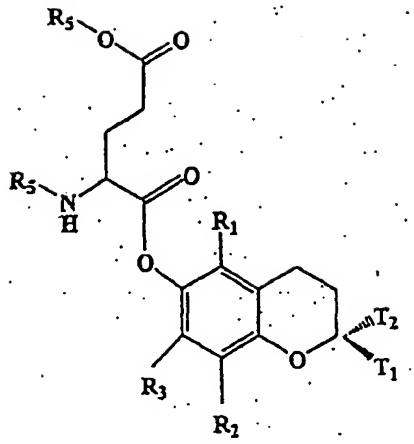


FIG. 10

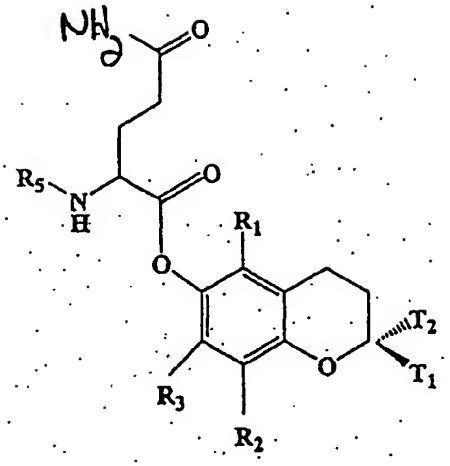


FIG. 11

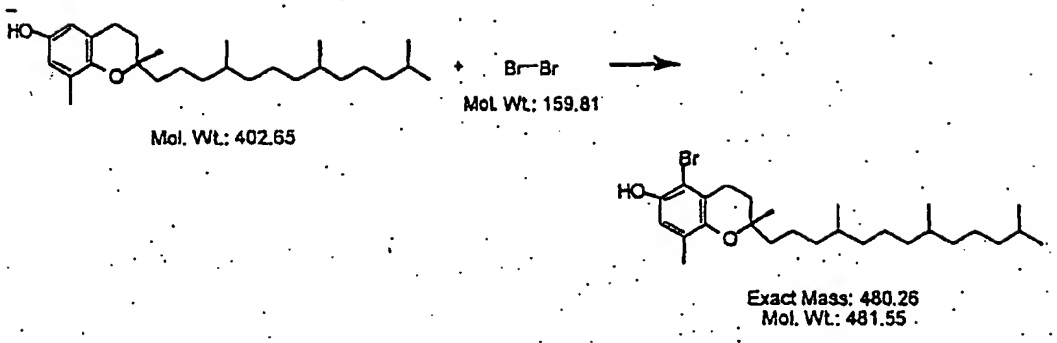

HO
Br—Br
Mol. Wt.: 159.81
Mol. Wt.: 402.65
Br
HO
Exact Mass: 480.26
Mol. Wt.: 481.55

FIG. 12

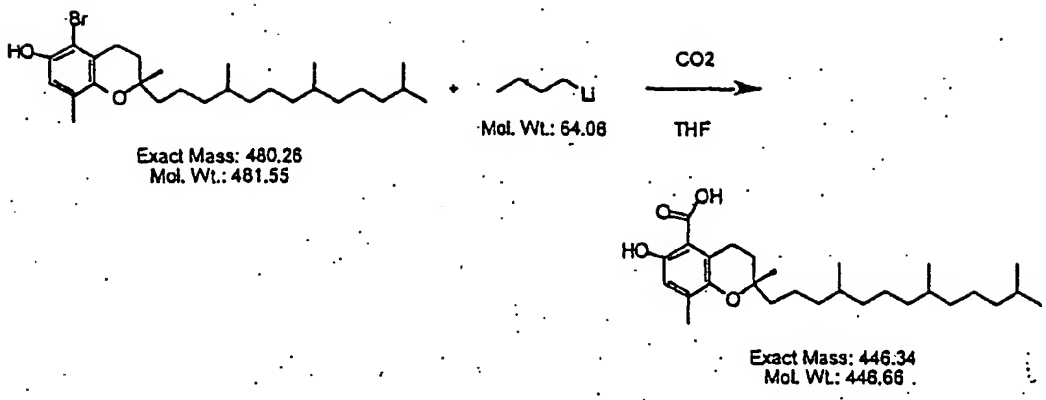

Br
HO
O
Li
Mol. Wt.: 64.06
CO2
THF
Exact Mass: 480.26
Mol. Wt.: 481.55
OH
O
HO
O
Exact Mass: 446.34
Mol. Wt.: 446.66

FIG. 13

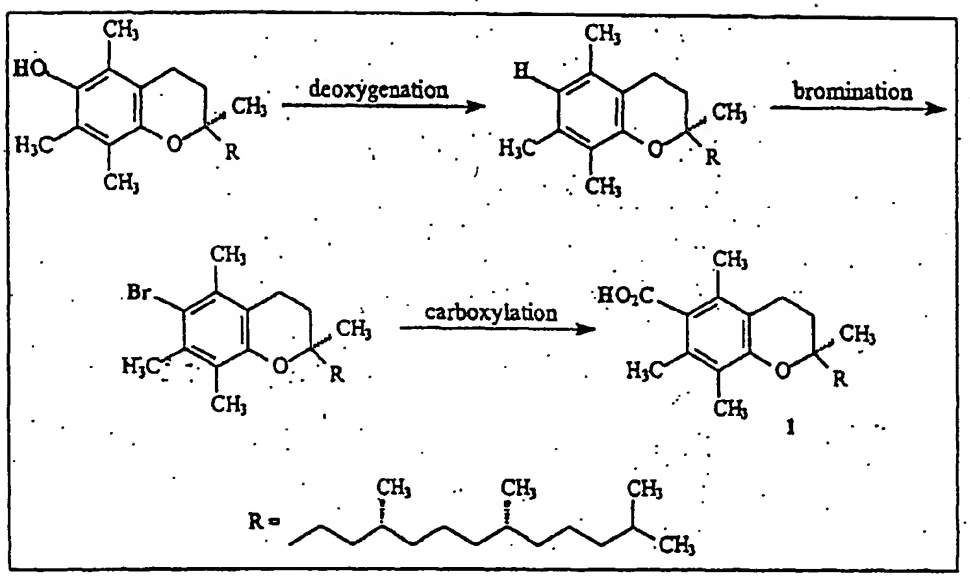


FIG. 14

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
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(54) Title: CARBOXYBENZOPYRAN DERIVATIVES AND COMPOSITIONS

(57) Abstract: Benzopyran derivatives including carboxylbenzopyran derivatives are described. Compositions that include the benzopyran derivatives are also provided.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/11266

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C07D 405/06, 311/04, 311/22, 311/72
US CL : 549/405, 407, 408, 410
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 549/405, 407, 408, 410

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE, CHEMPAT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	US 6,387,882 A (OGATA et al) 14 May 2002, column 1 lines 50+, claim 1.	1, 3, 11
X	US 5,917,060 A (ROSENAU et al) 29 June 1999, column 3, lines 20-46.	1, 8, 14, 18, 19
X	US 5,534,536 A (OHUCHIDA et al) 09 July 1996, column 5 lines 30+, column 2 line 1-24 and claim 1.	1-21
Y		1-21
X, P	US 6,239,171 A (LANE et al) 29 May 2001, column 9 lines 36+, column 10 lines 1-20.	1-21

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